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IXODES RICINUS IN RELATION TO ITS PHYSICAL ENVIRONMENT

IV. AN ANALYSIS OF THE ECOLOGICAL COMPLEXES CONTROLLING DISTRIBUTION AND ACTIVITIES

BY JOHN MACLEOD

Cooper Technical Bureau, London

(With Five Figures in the Text)

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I. INTRODUCTION

NUMEROUS records exist of the occurrence, both regional and seasonal, of the tick, *Ixodes ricinus*. Records of its world distribution have been collected by Nuttall (1911, 1916), Senevet & Rossi (1926) and Olenev (1929, 1934, 1934*a*). In 1932, the writer recorded detailed observations on the seasonal and regional distribution of the tick in Scotland. In previous papers of the present series (MacLeod, 1934, 1935, 1935*a*), the relation of the tick to its physical environment has been discussed in detail, studies being made of the factor complexes governing survival, activity, reproduction and development. From the results obtained under laboratory conditions, it should now be possible to interpret to

some extent the observed phenomena of seasonal and local distribution in terms of the underlying causes, and to outline the probable limits of potential world distribution of the species.

It is well known that life in general is intimately affected by meteorological factors, which, by their combined effects, impose the ultimate limits to the distribution and activities of most free living organisms. Imms (1931) defines the relationship as follows:

"Climate may be defined as the complex of meteorological factors prevailing throughout the year for a given area, while the incidence of these same factors at some particular point of time constitutes weather. Climate, in the broader sense, influences the geographical and ecological distribution of species, while weather influences their local prevalence by seasons: the one operates in space, the other in time."

With the tick, however, the position is complicated by its parasitic habit of life; the evolutionary adaptations which usually accompany the habit might be expected to modify the application of this general principle, and a survey of the biotic relationships of the tick becomes necessary.

II. The influence of the biotic environment

The local and geographical distribution of the tick would *a priori* be expected to depend primarily on biotic factors. As a parasite, it is dependent on the presence of its hosts, and enemies, both predaceous and parasitic, might be expected intimately to affect its numbers, since it is exposed to their attacks both in its parasitic and in its free-living phases.

(*a*) *Hosts and world distribution*

A study of the rôle of alternative hosts (MacLeod, 1934*a*) showed that the presence of the chief host, the sheep, is not necessary to the continued existence of a tick population: even a restricted range of alternative hosts was able to maintain it at an appreciable level. When the possible alternative hosts from which the tick has actually been recorded are considered, it is seen that this species has a host list of extraordinary catholicity, ranging from subtropical lizards to the Arctic fox, embracing carnivores, from the polecat to the puma, herbivores of such widely differing geographical range as the red deer and the camel,[1] rodents, and a wide range of birds, varying from predaceous falconids and owls to the sportsman's game birds, and even to the unlikely cormorant. The geographical distribution of the species, therefore, is not likely to be intimately dependent on the host factor. Actually, the northern limit of occurrence, as judged from available records (*vide infra*), falls short of the limits of forest and agriculture; that is, the tick range does not appear to extend to the limits of distribution of its possible hosts. It would appear, therefore, that

[1] Reported from the camel by Smith (1930). For other hosts see Nuttall (1911) and MacLeod (1934*a*).

physical rather than biotic factors govern the world distribution of the species.

(*b*) *Enemies in relation to local and seasonal prevalence*

The possibility remains that local distribution or seasonal prevalence in a physically favourable area might be governed by biotic factors, such as the presence of a particular predator or parasite. The only known parasite of this species, however, is *Hunterellus hookeri* (*Ixodiphagus caucurtei* du Buysson), which has been recorded from *Ixodes ricinus* in France. Cooley & Kohls (1934) have studied the possible establishment of this parasite in the United States of America, and have come to the conclusion that, in the Transition Life Zone, only one generation a year is possible, although conditions may be more favourable for its multiplication in the Upper Austral Life Zone. If only one generation per year were possible, the biology of the parasite, in outline, would be that the eggs would over-winter in parasitised ticks, the larvae develop in late spring and summer and the adults emerge in autumn to parasitise the ticks then appearing. Even were two generations of the parasite per year possible, the degree of dispersal of this species of tick in its natural habitat is such that a sufficiently high percentage of parasitisation is improbable, since the principal hosts, herbivores, do not tend always to return, like carnivores, to the same haunts.

There is no evidence of the effect of predators on the free-living tick population, though, doubtless, many fall victims to predaceous insects, arachnids and birds. Field-mice also probably devour ticks, especially the large gorged females. During its parasitic phase, the tick is protected from the attacks of predators, with the possible exception of the starling.

From the foregoing review, it would appear that the tick is singularly fortunate as regards its ecological niche; it does not suffer markedly either from individual or interspecific competition, and it occupies the happy position of a loose end in the food chains of the animal community, being comparatively free from parasitism, and, because of the absence of any gregarious tendency in the non-parasitic phase of the nymphal and adult stages, being largely insusceptible to devastating attack from predators. The significant variables in its environment, unlike those of most predators, would, therefore, appear to be physical rather than biotic.

An interesting speculation in this connection is that which concerns the nature of the mechanism regulating tick numbers in nature. The potential increase of ixodid ticks generally is enormous: Lounsbury (1899) estimated 20,000 as the maximum number of eggs deposited by a female *Amblyomma hebraeum*, and the average deposition of 18 species, based on the records of 151 individuals given by Hooker, Bishopp & Wood (1912), is 3120 eggs per tick.

The average deposition of *Ixodes ricinus* females under optimum conditions (MacLeod, 1935*a*) is about 2000 eggs. Since the ratio of females is 9 : 5

(Macleod, 1932), it follows that in a stable population $1 - 14/9 \times \frac{1}{2000}$ or 99·922 per cent. of each generation succumb to the vicissitudes of their environment. The normal regulating mechanisms of animal numbers, *e.g.* upsets of the balance of food chains, intraspecific competition, checking, either facultative or obligatory, of reproduction, and so on, are unlikely to play any important part here, under normal conditions. Thus, the dispersal of the tick during reproduction prevents the likelihood of density producing psychological or physical checks on reproduction, competition is practically absent because of the numbers and size of the available hosts, and the tick while on the host is comparatively immune from attack. The only likely biotic controls are predation during the free phase, and failure to effect parasitisation.

Apart from these, the eliminating factors will be physical,—desiccation, drowning, thermal extremes, accident of mechanical injury, and so on. These are uninfluenced by fluctuation of population numbers, and are not therefore automatic checks. The nature of the factor or factors maintaining equilibrium remains obscure.

III. Seasonal periodicity

It has been shown that there occur, in Scotland, two distinct periods of tick activity, one in spring, the other in autumn. Wheler (1899) and Brumpt (1922) record a similar periodicity in England (Alnwick Park) and in France.

Wheler (1899) suggested that the explanation of this phenomenon lay in the mildness of the climate, which allowed of the rapid development of the spring-fed ticks, and their engorgement again in the autumn of the same year.

This explanation assumes that all the unfed ticks become engorged in spring, and does not take cognisance of the fact that the spring period of activity terminates at approximately the same time (early June), irrespective of whether the ground is heavily or lightly stocked with sheep.

(*a*) *Cyclical diapause and winter inactivity*

Falke (1931) explained the winter cessation of activity as due to the onset in autumn of a state of torpor from which the tick does not recover until spring, irrespective of weather conditions. This theory was supported by Totze (1933). Such an attunement of causally unrelated happenings (Roubaud, 1922) or dissociation of originally related happenings (Cousin, 1932; Dawson, 1931) is apparently possible: these workers have produced evidence suggestive of the existence in certain insects of a synchronisation of hereditary rhythm with external periodicities. Wigglesworth (1934), however, regards the problem as one of growth, hereditary diapause being induced by the failure of growth-promoting hormones. If this be so, suspended respiratory metabolism, in an unfed tick, cannot be properly considered true diapause. If the phenomenon be admitted to include arrest of motility, then, in the case of the tick, the onset of the cold season should serve merely to liberate the stimulus to diapause, and

cold weather should not be necessary to maintain it. Totze suggested that this actually is the case, but the writer's experiments (1934, 1935) fail to support his hypothesis, and it is more probable that the winter inactivity of the tick is a simple reaction to the prevailing low temperatures. In support of this is the fact that cases of louping-ill, a tick-transmitted disease, have been recorded during mild spells in winter, indicating some degree of tick-activity.

(*b*) *Temperature and activity*

The writer has shown (1932) that there is a close correlation between air temperatures and degree of tick infestation of sheep, and also that infestation

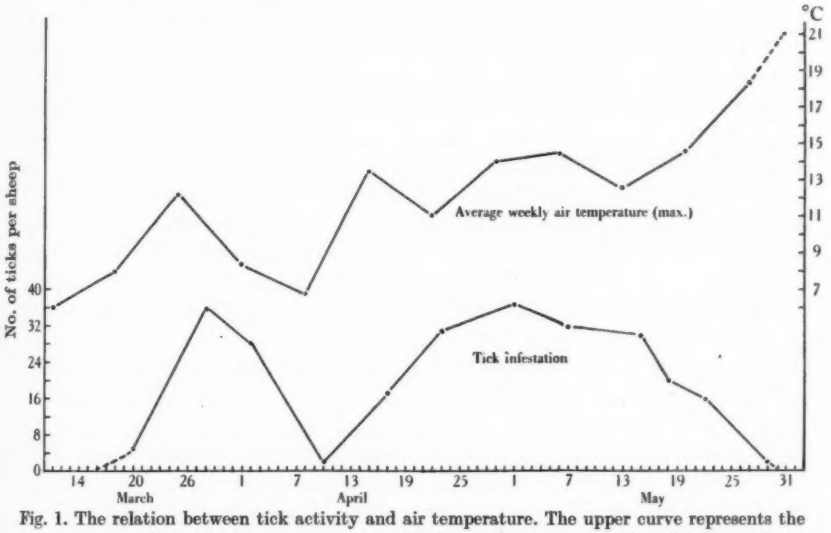

Fig. 1. The relation between tick activity and air temperature. The upper curve represents the average weekly maximum air temperature; the lower, the average number of ticks per sheep. Activity commences when the temperature exceeds 7° C., and practically disappears when it exceeds 16° C.

occurs when the average maximum air temperature lies between the approximated limits of 7 and 15–16° C. Confirmation of the existence of this correlation is illustrated in Fig. 1, which shows the variation in tick infestation from mid-March to the end of May 1934 on sheep in the Callander district. The weekly averages of the daily maximum temperatures for the nearest meteorological station (Perth) have been plotted on the same figure. Tick activity commenced when the temperature rose above 7° C. and fell to a negligible level when it exceeded 16°C. In early April, there was a return to winter conditions, and the figure shows a corresponding drop in the degree of infestation for this period.

It does not necessarily follow from this correlation that air temperature governs activity; evidence of a causal relationship, however, is forthcoming

from a study of the geotropic responses of the tick, which showed (MacLeod, 1935) that temperature determines the quality of the tick's response to the stimulus of gravity, the tick being negatively geotropic at temperatures of 14–24° C., while, above and below these limits, the response is positive. The apparent discrepancy between the observed and the experimentally determined limits is readily explained by the fact that air temperatures refer to the shade temperatures of the air, and are not comparable to the temperature of an object which is exposed to the radiant heat of the sun. Thus, in a tick exposed on the tips of vegetation, an upper limit of 24° C. would easily be reached by insolation when shade temperatures were in the neighbourhood of 15–16° C. Again, as regards the lower limit, it must be remembered that the elements of a microclimate may differ markedly from those of the macroclimate. Thus, Tutin (1928) has shown that the mean air temperature at Long Ashton during the greater part of the year is lower than the soil temperature at a depth of 4 in., the difference sometimes being as great as 20° C. This difficulty of relating events in the lives of insects to climatic happenings, as expressed in official meteorological data, is a serious one in most cases, since the majority of insects frequent specialised microclimatic habitats. The difficulty constitutes, in the writer's opinion, an outstanding objection to the use of bioclimatic maps, since the relationship between micro- and macroclimate will not be constant, but will vary both in time and space, and with edaphic factors in any given area.

Since the response of the tick to geotropic stimulus varies with temperature, it follows that temperature determines the relative accessibility of parasite and host; a tick at the roots of vegetation is obviously less likely to be picked up by a passing host than one on the tips. If the observed limits of activity, 7 to 15–16° C., be taken as the air-temperature equivalents of the actual limits for negative geotropic response, it becomes possible to explain the seasonal nature of tick activity as a temperature effect; at winter temperatures below 7° C. the tick is at the roots of vegetation; at temperatures between 7 and 16° C., *i.e.* spring and autumn temperatures, it climbs to the tips of the vegetation and so becomes readily accessible to hosts; while, at temperatures over 16° C., summer temperatures, the tick, being again at the roots of vegetation, is less readily picked up. It has been shown, however (1935), that the temperature range for normal activity extends beyond the upper limit for negative geotropism; that is, at air temperatures over 16° C., the tick may still be active. Thus, if a host should lie down on a tick, or, in browsing among grass roots, should come in contact with one, attachment could readily be effected. Thus it is that occasional ticks may be found on hosts throughout the summer months.

The "double-attack" type of activity should be characteristic of countries with warm summers and cold winters, with fairly long transition periods between these extremes. It follows that in colder countries, where the maximum summer temperatures do not exceed 16° C., there should be only one period of activity, a summer one, while, in warmer lands, tick activity should occur, if at

all, only in winter. A study of the available records of seasonal occurrence reveals that this appears to be the case. Thus, in central Russia, the ticks are found from May ("before the trees bud") to September (Olenev, 1927), while, in Algeria, they appear from November to April (Senevet & Rossi, 1924).

(c) Life cycle in nature

It has been shown in previous papers of this series that there are different temperature optima for activity, reproduction and development. The probable duration of the life cycle in nature must therefore be studied in relation to the seasonal occurrence of these optima; mere summation of the time required for completion of each stage is of only theoretical value. Various results have been obtained by the latter method. Falke (1931) gives 182 days, Elmanov (1930) 180–1874 days, Olenev (1927) 285–2194 days, Nuttall (1911) 178–2724 days, "about 170 days probably represents the shortest possible time required...", Stockman (1916) 160 days (spring eggs), 290 days (summer eggs), Wheler (1899) 33½ weeks.

The following experimental data allow some conception to be formed of the probable sequence of events in nature. The temperature range for parasitisation is 14–24° C., whereas that for development is 15–35° C. Oviposition can proceed at temperatures of 10–27·5° C., and can commence in 8 days after engorgement of the female. Translating these facts into terms of seasonal variations in Britain, one finds the tick functioning as a parasite under spring conditions of moderate temperature. High summer temperatures are unsuitable for active parasitisation, which is consequently infrequent, but this season provides the optimum conditions for development of the ticks gorged in spring. Many of these are ready, therefore, to act as parasites again by the time suitable conditions are provided in the moderate temperatures of the autumn. The winter is passed either in the gorged state, or, where the ticks failed to parasitise a host during the short autumn season, in the unfed state. Those ticks which hibernate as unfed ticks appear at the beginning of the spring tick-season. The autumn-gorged ticks develop slowly during the late autumn and early spring months, whenever the temperature of the microclimate exceeds the developmental threshold of 15° C. Gorged larvae and nymphs can complete development in 3 months at a temperature only 5° C. above the threshold (MacLeod, 1934); during the 5 months' period, mid-October to mid-March, there are, normally, sufficient days with temperatures over 15° C. to allow of development being well advanced by March. Under the increasing temperatures of March, development is completed, and the resulting unfed ticks enter a parasitisation phase under the favourable conditions of spring (Fig. 2).

Winter and summer may, therefore, be regarded as development seasons, spring and autumn as parasitisation seasons. The alternation in the seasons is repeated in the life-history of the tick—unfed larva, developing larva, unfed nymph, developing nymph, unfed adult. An apparent difficulty arises in the

fact that the active adult phase is followed by two successive passive phases, that of the gorged female and of the egg. It is to be remembered, however, that the temperature range for ovulation, 10–27·5° C., corresponds more closely to the range for activity than to that for development. Also, under favourable conditions, the pre-oviposition period may be as short as 8 days, and seldom exceeds 30 days. Therefore, adult females, engorging during a parasitisation season, find in that same season the conditions favourable to oviposition, and can generally complete the process before the seasonal limit is passed. The

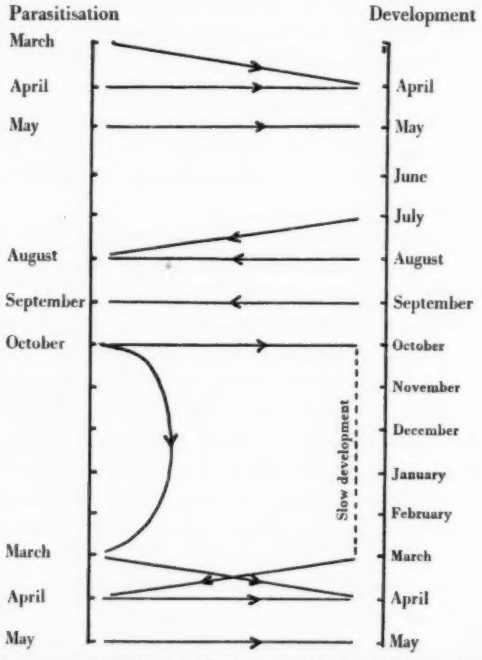

Fig. 2. The relation between the seasons and the appearance of unfed ticks. Spring-fed ticks enter on development in April-May and become ready to parasitise hosts from July to September. Parasitisation is effected in August–October or, failing that, in the following March. In April appear these ticks which fed in autumn and developed during October–March.

shortness of the autumn season is compensated by the fact that oviposition can proceed at temperatures down to 10° C. Thus, the phases of parasitisation and oviposition in the female may be regarded as one, requiring moderate temperatures, and so the alternation of phases remains uninterrupted throughout the life history.

It would thus appear that, provided hosts are readily available, the life cycle requires a parasitisation—and a development—season to each of the three stages, *i.e.* at least six seasons, or 1½ years, for completion. The cycle is illustrated in Fig. 3.

Actually, there will be a wide variation in the time taken by different individuals to complete their cycle, according to the winter temperatures and to their success in finding suitable hosts. Since each unfed stage can survive at least a year, the duration of a particular cycle may be at least 3 years more than that outlined above, *i.e.* it may extend to $4\frac{1}{2}$ years, or even longer.

IV. HABITAT REQUIREMENTS

The problem of the causes underlying the local distribution of the tick is simplified by the habit of feeding it has adopted. Being dependent for dispersal on the will of its host, it can exercise no selection in relation to its habitat, and its physiological requirements, rather than any possible reflex reactions to habitat conditions, will determine its actual distribution.

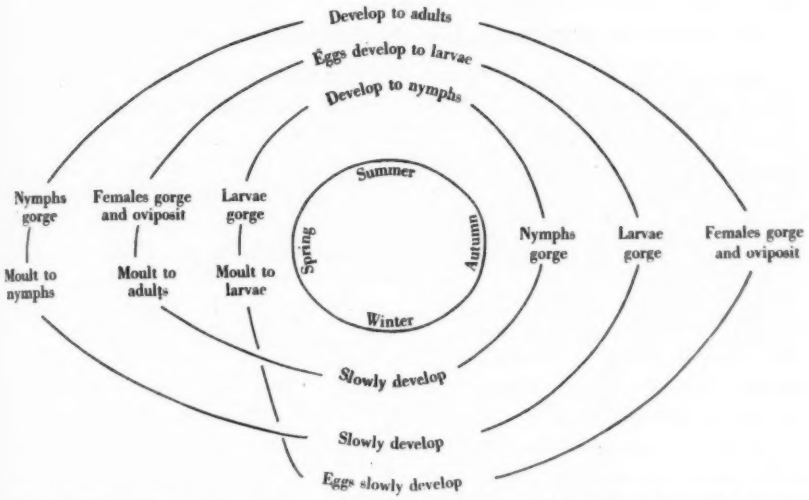

Fig. 3. Illustrating the seasonal life cycle of the tick. The cycle is $1\frac{1}{2}$ years, whether the generation starts with spring or autumn eggs.

Many observers record that the tick is most commonly found in swampy, undrained areas, or where a coarse vegetation of shrubs, bracken or heather is present. Olenev (1927), from a study of the moisture requirements of the tick, was able to show that this choice of habitat was due to the high degree of humidity necessary for its survival and development. His conclusions have been confirmed by the writer, who showed (1935) that a relative humidity of over 75 is necessary for survival, reproduction and development at normal temperatures. Under conditions of arable pasture such high humidity in the vegetation does not obtain throughout the year, the underlying soil frequently drying out in the top few inches during the summer. In swampy areas, however, where the water-level of the soil remains more or less at the surface

throughout the year, evaporation is never checked by water scarcity, and, providing there is a layer of vegetation to furnish a buffer stratum of still air between the wet soil surface and the outer atmosphere, a saturated, or almost saturated, microclimate is maintained even in summer. Thus the factors which will determine the suitability of a habitat are primarily edaphic, the nature of the soil, the presence or absence of a humus-forming layer, and the vegetation type. In Britain, these requirements are generally satisfied by hill sheep pastures: the soil is frequently peaty and, therefore, moisture-holding; the vegetation is rank or coarse, and a mat is formed of the withered vegetation of the previous year. In such conditions the tick finds its ideal home, sheltering, in winter and for reproduction and development, under the mat of decaying vegetation, climbing, in spring and autumn, the stems of *Nardus*, rushes, heather or bracken, to find its host.

(*a*) *Comparison of different habitats*

Measurements of the air humidity in different types of hill pasture were made in the spring of 1934, by means of a hair hygrometer. Three types were chosen: (1) old permanent pasture, with moss and tussocks of coarse grass—a common type of hill pasture; (2) ground inclined to be wet, but not strictly marshy—the vegetation layer consisting of old grass, rushes, etc.; (3) marshy land, the vegetation type being chiefly rushes. The three areas were of small extent and intermingled; the actual dispersal of ticks over them may, therefore, be taken as fairly uniform.

The following were the humidity readings for each type, and the corresponding air humidity, as recorded by the same instrument:

	Type 1	Type 2	Type 3	Air
April 18	70	100	100	60
24	87	95	100	56
May 2	89	99	100	65
12	78	98	100	58
18	75	78	97	60
29	87	84	96	62

The relative number of ticks on each type of habitat was roughly estimated by dragging a woollen blanket for a fixed distance (50 yards) over the herbage, and counting the number of nymphs adhering to the blanket. The results were as follows:

Type	No. of counts	Average no. of ticks per count
1	10	2·7
2	10	4
3	6	13

The result is open to objection since the counts were made over 2 or 3 days, and the number of ticks on the grass tips would vary with the air temperature; the evidence, such as it is, however, tends to support the general thesis of the dependence of population density on edaphic factors, where the host factor is constant.

It is clear that summer must be the critical time, in Britain, for survival of the tick, since at that season the humidity in the vegetation is most likely to decrease through drying of the soil. Many arable pastures afford sufficient cover and moisture for ticks in spring and autumn. Ticks introduced in autumn to such pastures by birds or brought in on hill sheep establish themselves as individuals, moult, and may even engorge the following spring; yet, because of the summer drought, establishment as a species does not occur. Thus it is that, in spite of the frequent movement of sheep from hill to lowland pasture, the tick remains confined to the hill pastures (see MacLeod (1932) for distribution in Scotland).

(b) Spread of species in relation to ecological succession

If an arable pasture be allowed to deteriorate to such an extent that the soil becomes acid and moss begins to choke out the grasses, conditions become increasingly favourable for the tick. On such pastures, as the mortality due to climatic causes decreases with changing vegetation type, dispersal is followed by individual establishment, and individual establishment gradually merges into the establishment of the species. Two actual instances of this sequence were brought to the writer's notice, where the ecological succession of the vegetation in old permanent pasture allowed of ticks establishing themselves in lowland areas. In one of these cases the farmer had been in the habit of renting his pasture as grazing for hill sheep in spring. Ticks were thus constantly being introduced, but previously had failed to survive, and fresh stock was put on these pastures in autumn with impunity. Latterly, however, the autumn-grazed herds suffered increasingly severe epidemics of tick-borne fever when introduced to this pasture. Examination of the sheep revealed the presence of engorging ticks; these had dropped in spring from sheep infected with tick-borne fever, had survived the summer, and were now spreading the infection to the non-immune lowland herd. Severe harrowing of the pasture to tear up the moss and heavy liming to lower the acidity were recommended as the obvious curative measures.

V. WORLD DISTRIBUTION

(a) Some general considerations

On the basis of results obtained in the laboratory, many attempts have been made to map, from meteorological data, the potential areas of distribution of different insects. The application of meteorological data in this manner is, however, on account of its misleading simplicity, full of pitfalls for the unwary, and a clear understanding of the underlying theory of climatology is necessary to a proper appreciation of the biological significance of any given set of data. One difficulty already mentioned is that of referring the macroclimatic values of a given area to specialised microclimates or even ecoclimates in that area. A further difficulty in the adoption of standard meteorological data in biological work is that these are given as means, and thus tend to obscure the extremes,

although the latter intimately affect life, especially in the case of animals living near their geographical limits. With world isotherms, again, the temperatures employed for plotting these are reduced to sea-level in the ordinary type of isotherm map. The actual temperatures along such isotherms may differ markedly from the given value, on account of varying altitude; therefore, from the biological point of view, such maps may be quite misleading.

It has been shown that the conditions necessary for survival of the tick are represented by a complex having its origin in the interaction of macroclimatic and edaphic factors. Since climate type is not determined by any one factor alone, it is obviously impossible to define this complex in terms of one factor; the distribution of the tick must, therefore, be considered in relation to the climate as a whole, bearing in mind that, generally speaking, the vegetation type will be conditioned by climate.

In any given climatic type, distribution will be determined partly by the separate action of each constituent factor, and partly by the interaction of the different factors. Thus, rainfall is an important factor determining the humidity of the microclimate, but this will also depend, to some extent, on the rate of evaporation. Therefore, the velocity and direction of the prevailing wind must be considered, for the value of the wind as a desiccating agent will depend largely on whether it has travelled over land or sea. Again, the nature of the soil is important: a porous soil will absorb heavy rain without leaving any moisture accessible at the surface, while a heavy soil will result in abundant microclimatic humidity with a much lesser amount of rain. The geological factor even will affect the influence of rainfall, *e.g.* an impervious stratum will allow of marshy areas forming, as in the Roman Campagna, where the impervious volcanic floor has resulted in the stagnation of surface water even under moderate conditions of rainfall.

Temperature also will have important intereffects with other physical factors. Soil type will affect the response of the ground, and, therefore, of the microclimate, to temperature changes. High precipitation, where the temperature is below freezing, will ensure a snow blanket, the biological significance of which is well known. Sunshine will affect the ticks both directly by insolation and indirectly by radiation of heat from the ground; the degree of cloudiness will affect the value of insolation, a result of great significance at high altitudes, where, although shade temperatures are greatly reduced below those at sea-level, the ground temperature under the rays of the sun may rise very considerably.

The various intereffects of this nature must be taken into consideration as well as the direct effects of separate factors, in discussing the suitability for the tick of different geographical areas for which meteorological data are available.

(*b*) *Latitude and distribution*

Senevet & Rossi (1926) summarised the existing evidence on the distribution of *Ixodes ricinus* and concluded that the species is a tick of cold or temperate climates. They found that, with a few exceptions, the areas from which

the tick had been recorded were north of the 40° parallel of latitude. In a few instances the tick occurred between this parallel and the tropics, and they suggested that in these regions development was inhibited by the summer heat and occurred only in winter, whereas, in temperate regions, it occurred in spring and autumn. Their suggestion arises from an understandable misconception of the seasonal cycle of events; activity and development have been assumed to be synchronous. It has now been shown, however, that development occurs at temperatures above those necessary for activity, and can proceed at temperatures up to the limit for survival.

While latitude undoubtedly plays an indirect part in determining the distribution of a species, any single parallel can seldom be expected to represent a limiting factor. The 40° parallel traverses the continental land interiors and margins, and the climatologically unique Mediterranean basin; the land and sea distribution of the latter region has superimposed on the fundamental planetary régime along this latitude a complex variety of climatic types, which, collectively, differ from that found at the same latitude in the interiors and on the simpler marginal regions of the Pacific and West Atlantic. This parallel obviously cannot be considered a bio-climatological unit.

Similarly, the northern limits of distribution are unlikely to be represented by a single parallel, since the relief of the land masses in the northern hemisphere results in wide latitudinal convolutions of the isotherms, these bulging equatorwards in the lee of mountain ranges which impede the warmth-bearing westerly winds, *e.g.* eastwards of the Rockies and the Urals, and being forced polewards over the oceans. Olenev (1934) points out that the northern limiting zone of distribution of *Ixodes ricinus* lies generally between the 50° and 60° parallel, except between the 30° and 40° meridians, where the species has been recorded further north; he recognises, however, the importance of climate, among other factors, in determining the distribution of tick species.

(c) *Limits determined by temperature*

Obviously, the two chief single factors affecting the tick are temperature and moisture. Since the moisture requirements are relative to the maximum moisture-holding capacity of the air and not absolute values of vapour pressure, it follows that the conditions could be satisfied at almost any latitude. In contradistinction to moisture, temperature is largely governed by latitude and will, therefore, be the fundamental limiting factor to distribution. Within the geographical limits set by temperature, distribution will be governed by the climate complex, the principal factor in which is precipitation.

The actual microclimatic temperatures critical for the tick, as determined by laboratory experiment, are as follows:

	Temp. °C.
Survival (1 day)	(−14 to −12) to 35
Parasitisation	14 to 24
Oviposition	10 to 27·5
Development:	
Eggs	15 to 30
Gorged ticks	15 to 35

It is clear, therefore, that, as regards temperature, survival of the species will depend on (1) the extremes in the microclimate not surpassing −14 and 35° C., (2) the temperature remaining below 24–27° C. sufficiently long to allow of parasitisation and oviposition, and (3) the temperature remaining for a sufficiently long period at a value over 15° C. to allow of development.

As has already been pointed out, it is not possible in the present state of our knowledge to interpret accurately these limits in terms of air temperatures. The limits for parasitisation in Britain were found to correspond to air temperatures of 7–16° (average weekly maxima). The mean temperatures of the months during which this range occurred varied from 5 to 10·5° C. It is by no means likely that these same weekly or monthly air temperatures would correspond to the same microclimatic temperature-range in different countries. Bearing this objection in mind, however, an approximate index to microclimatic conditions can be obtained by using the mean monthly isotherms. Thus, the isotherm of 10° C. for the coldest month should represent, approximately, the equatorward limit of possible distribution, since at higher mean monthly temperatures parasitisation will not readily occur. Similarly, subject to a variable correction, which will often be of great magnitude, for temperatures at the soil surface, the isotherm of 95° (mean maximum) for the hottest month will determine the extreme equatorward limit of survival. The correction factor, however, varies both in space and at different times in the same area. Smith (1929) records observations at California over two successive periods of February–December and December–June. Air temperatures were compared with temperatures ½ in. below the soil surface; the ratio in the first period was 86 : 114° C., yet in the second only 81 : 76°C. On one particular date, when the air temperature was 46–47° C., the temperature just below the soil surface was 61–62° C.

Mackenzie Taylor (1928) studied the relation between soil and air temperatures at Giza (Egypt) and found that a regular relationship could not be traced between them. The maximum temperature at the soil surface was always considerably above the maximum air temperature, the difference in the mean monthly maxima in July being as much as 25° C. He records surface temperatures for July of over 60° C.

As regards the poleward limits for survival, the difficulty arises that the temperature of the air bears little relation to that of the soil surface when the ground is covered by snow. This dissociation is well illustrated by Mail's (1932) observations in Montana: during one winter when there was an almost constant covering of snow, the air temperature ranged from −25 to +8° C., yet, throughout the whole period, the minimum soil temperature did not go below −4° C. The effectiveness of freezing temperatures in limiting distribution will, therefore, be affected by the time of occurrence and the duration of the snow-coat.

The poleward limit of distribution in relation to temperature will, however, also depend on the duration of soil temperatures over 15° C. An approximate

idea of the relation between soil and air temperatures in cool temperate regions can be obtained in the following manner. It was found (MacLeod, 1932) that eggs placed at the soil surface outside during the period May–July hatched in 9–10 weeks. The mean monthly air temperatures for these 3 months in central Scotland range from 10 to 15° C. Now, it had been shown (MacLeod, 1935*a*) that, under laboratory conditions, 9–10 weeks is the period taken for development of eggs at 14–18° C.

A comparison of the time taken for development of eggs and of gorged ticks at several temperatures shows that the nymphs take about two and a half times as long to develop as the eggs, thus:

	17–23° C.	21–23° C.	25–26° C.
Eggs	5 weeks	30 days	24 days
Nymphs	13–24 „	78 „	61 „

Therefore, at 14–18° C., nymphs would require over 20 weeks to develop, that is, 5 months with mean air temperatures of 10–15° C. Allowing a minimum period of 3 months with mean temperatures over 10° C. for development in cool climates, that is, June, July and August in the northern, December, January and February in the southern hemisphere, the isotherm of 10° for June and December respectively can safely be regarded as including the furthest poleward limits of distribution of the tick.

Fig. 4 shows the tentative temperature limits of distribution so far defined. Olenev (1934) directs attention to the partial coincidence of the northern limits of distribution of tick species in general with the July isotherm of 15°. The agreement, however, is not complete, as this isotherm excludes Scotland and northern England, British Columbia and other regions where ticks occur.

(*d*) *Limits determined by precipitation*

The maintenance of high humidity in the microclimate depends on a number of factors other than rainfall: dew and mist may maintain almost saturated conditions, and yet have little appreciable effect on the recorded precipitation. Further, the actual rainfall will vary in effectiveness according to its nature and distribution; torrential downpours may result in a high rainfall value in a short period, yet much of this rain may be lost by surface drainage.

It is improbable that rainfall can set an upper limit to distribution of the tick, since the species is extremely resistant to actual submersion (see MacLeod, 1935). Vorontzev (cited by Hooker, Bishopp & Wood, 1912) states that eggs of this tick can survive under water from autumn to spring. With regard to the lower limit of rainfall, even were its distribution and nature uniform, the amount would not be a constant, but would vary to some extent with temperature, since less is required to produce saturated conditions, and the rate of loss is less at low temperatures than at high. The rate of loss by transpiration will depend on the type of vegetation, forest transpiring more rapidly than grass.

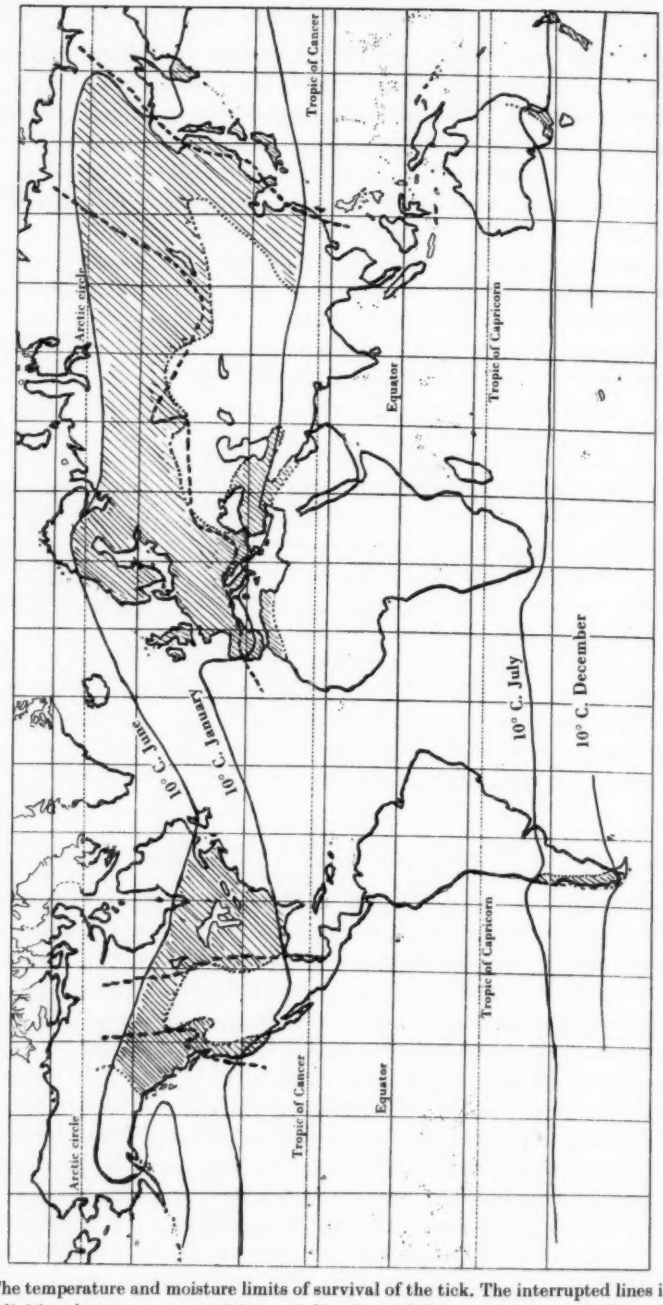

Fig. 4. The temperature and moisture limits of survival of the tick. The interrupted lines indicate the divisions between areas receiving periodic rains and those receiving rain at all seasons. The areas having a forest type of vegetation are lightly shaded.

So far as precipitation is concerned, no precise limits can be set to the areas favourable for survival of the tick.

Generally speaking, climates with periodic rains, *i.e* with regular dry seasons, should be unfavourable to the tick, as compared with climates experiencing rain at all seasons. On Fig. 4, interrupted lines have been drawn across thermally possible areas of distribution, separating the two types of rain distribution.

The type of vegetation plays an important part in determining the effectiveness of rainfall by affecting the rate of evaporation from the soil to the air. In turn, it is determined mainly by the distribution and amount of rain: forest requires a constant supply of moisture, prairie grass is adapted to seasonal drought, while xerophilous desert plants clearly indicate more or less continuous drought. In the absence of detailed weather records, therefore, probably the most accurate index of the climate type is afforded by the dominant type of vegetation, provided due allowance be made for human interference, as expressed in deforestation, irrigation, and cultivation.

The division between forest, including woodland, grass and cultivation on the one hand, and steppe, prairie, desert and tundra on the other, is indicated on Fig. 4. The southern limits of the forest type show some degree of correspondence with the hyetal equator which divides areas receiving periodic rain from those receiving rain at all seasons. The climate type indicated by forest and woodland is obviously that most suited, from the point of view of rainfall, to the tick. In this connection it is of interest that Olenev (1931) describes a zonal distribution of different species of ticks in the U.S.S.R., *Ixodes ricinus* being principally confined to the forest zone of the northern regions. The prairie and steppe lands, stretching in a broad belt through central Eurasia and limited northwards by the hyetal equator, indicate a type of climate generally unsuited to the tick because of the parching droughts associated with this vegetation type. Haviland (1926) describes the steppe as showing a surface of brown scorched grass in summer, "like a parched stubble field", a condition obviously unsuited for tick survival.

Between the forest and steppe regions proper, *i.e.* as determined by rainfall distribution, there occur certain areas which, although outside the rainfall limits, have a forest type of vegetation. The more important of these areas which fall between the prescribed temperature limits are the Mediterranean basin, the Caucasus region, the east of Lake Baikal, and the extreme east of Asia. The woodland type of vegetation in these areas indicates a marked modification of the "steppe" régime, either in the direction of amelioration of the desiccating conditions during the dry periods, or of interruption or reduction of these periods. No general statement can be made, therefore, of the probability of survival of the tick in these areas, and they must be treated individually.

(c) Possible limits of distribution in northern hemisphere

The areas of possible distribution of the tick have now been roughly delimited from the point of view of the two main climatic factors, temperature and rainfall. The climate of the different regions within these limits may now be briefly reviewed, and the northern hemisphere will be first discussed.

Generally speaking, western Europe, with the exception of the interior of Spain, should be suitable to the tick, because of its typical marine climate, resulting in high rainfall, fairly evenly distributed, absence of seasonal extremes of temperature and a gradual transition of seasons, affording the periods of moderate temperature necessary for parasitisation. The extreme temperatures and summer drought of interior Spain place this region outside the suitable limits.

The Mediterranean basin presents a complicated series of climatic types, resulting in an admixture of favourable and unfavourable areas, both north and south of the temperature limit defined in Fig. 4. Thus, a narrow strip of the coastline of Morocco, Algeria and Tunisia, limited landwards by the Algerian Plateau and by the desert, enjoys an "oceanic mediterranean" climate (Martonne, 1925), the temperature extremes being moderated to a feeble amplitude, rains occurring in autumn and winter, and the summer, although with low precipitation, having a high degree of air humidity. Thus at Casablanca the temperature of the warmest month (August) is 22·9° C., and although the summer is practically rainless, the relative humidity is 84.

The Greek peninsula, on the other hand, is unfavourable because of the pronounced high temperature and drought of summer. Five months have an average of over 20° C. and the mean maximum reaches 40° C. This climate type occurs on all the Aegean coasts and includes the climate of Asia Minor. The Syrian climate, an exaggeration of this continental type, having an extreme temperature amplitude, and absolute drought for 6 months, gradually merges from the Hellenic continental type to the desert climates of Mesopotamia and Iran. All the eastern end of the Mediterranean, therefore, with the possible exception of the immediate seaboard, should be unfavourable for tick survival.

Eastern Europe, so far as it falls within the limits already set, remains comparatively favourable for survival of the tick, since, except in the extreme east, the marine influence continues to be felt for long distances eastwards. The absence of high relief near the coast allows of the amount of orographic rain decreasing slowly eastwards, *e.g.* the 20-in. isohyet reaches to Moscow and Kiev. Similarly, the continental type of seasonal extremes in temperature has not yet established itself fully, except in the far east of Europe.

East of the Urals however, conditions are markedly different. Here several factors militate against survival of the tick. The continental type of climate is strongly exemplified in the temperature extremes. In Siberia there are recorded the lowest temperatures in the world, yet the summers are extremely

hot. The change from winter to summer and back to winter is not gradual, as in marine climates, but very abrupt. The tick, therefore, would be unable to effect parasitisation and oviposition before the excessive heat of summer or the cold of winter inactivated it. A second reason why this region is unsuitable for the tick is that under the influence of the winter high pressure centre here, precipitation is negligible—less than 1 in. for January. Snow will therefore be less heavy and its cover less constant than at the same latitudes elsewhere, and so the tick is not safeguarded from the lethal temperatures of −50° F. and even lower which occur here in the winter months.

The effect of the fierce summer heat of this region will be intensified by insolation, since the skies are more cloudless here than at the same latitude further west. The mean annual cloudiness, although largely determined by latitude, shows immediately east of the Urals and also in eastern Siberia, except on the coastal strip, a northern extension of clearer skies, while central Asia, from Asia Minor to Manchuria, has less than 50 per cent. of cloudiness. In southern Siberia, the conditions are somewhat modified. The extremes of winter low temperature are reduced, while on the other hand, the summer temperatures, in the foothills south of the Siberian basin, tend to escape the extreme maxima of the plains. In this region the rainfall type changes from rain at all seasons to periodic rains. Along the transition belt where forest and woodland merge into steppe the conditions should be favourable for survival over a narrow strip, limited northwards by temperature, southwards by precipitation.

The area east of Lake Baikal is outside the limits set in Fig. 4, until the eastern margin is approached. Here conditions are such that only a very narrow strip of the continental margin is suitable for the tick. The winter high-pressure centre over Asia induces a continental type of climate with severe extremes and a rapid transition. This type persists almost to the coast, as the constant monsoons prevent the influence of the sea making itself felt. Japan and parts of the coastline of Asia have a more moderate climate, the marine influence and the warm Pacific current inducing a temperate climate type, with well-distributed rainfall.

In western Canada and U.S.A., the mountain ranges close to the coast limit the marine type of climate, corresponding to the European, to a narrow coastal strip. The arid area of the Rockies should, generally speaking, be unfavourable for tick survival, except where locally advantageous circumstances, resulting from local relief, vegetation type and such, allow of favourable conditions occurring in limited areas. East of the 100° meridian roughly, the general type of climate is more suitable, from the point of view of rainfall. Winter extremes of low temperature of continental type occur, but their effect is to some extent modified by the almost constant snow cover. The Eastern States are comparatively favourable, except for the peculiar superimposition of a continental type of climate on the marine, resulting in occasional severe heat waves, during which lethal temperatures may be reached, and in irregular, severe spells of drought.

Alaska, though coming to some extent within the limits set in Fig. 4, is unlikely to favour survival of the tick because of the low temperatures prevailing over the peninsula, except for a narrow south coastal strip. Further, the interior has a very low rainfall, only 10 in. annually in some places. Winter temperatures are extreme in comparison with those of north European regions of the same latitude, since the Pacific, blocked at the Behring Strait, cannot influence the limit of Arctic conditions in Alaskan regions in the same manner in which the Atlantic, which has free access to the Polar Sea, affects the climate of northern European regions. In comparison with the positive winter temperature anomaly of 20–30° F. in north-west Europe, Alaska has a winter anomaly of −2° in the interior to 10° on the south-west coast. The low precipitation does not permit of a snow-coat effectively modifying the temperature extremes, and the low temperatures of winter therefore should make the Alaskan region unsuitable for tick survival.

The potential areas of distribution of the tick in the northern hemisphere have now been delimited in a general way. It is to be remembered, however, that the different regions have been grouped according to their general climatic facies; the limits outlined must be regarded as defining, in a broad sense, the major regions of distribution. The possibility remains that within a major region unsuitable to the tick there may occur limited areas where meteorological and topographic factors combine to upset the normal climatic type, and result in isolated potential loci of tick survival. Three regions in especial where the defined limits must be accepted with reserve are the Mediterranean basin, the central Asian plateau country, and the western American plateau. In the last two regions, the complexity of relief, high altitude and topographically determined diversity of vegetation type may readily result in comparatively favourable conditions occurring over limited areas.

Mountain areas on the equatorward side of the limits of distribution present a rather peculiar problem. Air temperatures are reduced, roughly 1° F. for every 300 ft. of altitude, but, above a certain level, rainfall also is reduced. There will therefore be a tendency to aridity, which will be intensified by the clearness of the air and other factors inducing an acceleration in evaporation rate. The nature of the rainfall also tends to be tropical in its periodicity, and the rain usually falls in heavy showers, thus losing much of its effectiveness. Up to a certain height, on the other hand, precipitation tends to increase with altitude, resulting in forest zones. Where these alpine forests occur, they will exercise a marked effect on the microclimate; the summer soil temperatures will be lowered and the extremes modified. Transpiration also will tend to reduce the temperatures in the ecoclimate, and therefore in the microclimate at the soil surface. It follows that no hard and fast rule can be laid down as to the suitability or otherwise of tropical and subtropical mountain regions. It may be said, however, that alpine forest zones in such latitudes indicate conditions possible for tick survival.

(*f*) *Possible limits of distribution in southern hemisphere*

In the southern hemisphere, the only land areas falling within the specified limits are New Zealand, except for the extreme north, the south-east corner of Australia, Tasmania, the southern Argentine and southern Chile. The New Zealand and Tasmanian climate, except for Auckland, is comparable to the English, the summers being cool and the winters mild. The rainfall in South Island especially is evenly distributed, and nowhere do real summer droughts occur. The rainfall in New South Wales, unlike that of most of Australia, is evenly distributed throughout the year, and the actual amount is fairly large. The annual range of temperature is comparatively limited, but sufficient to include parasitisation—and development—conditions; this area, therefore, should be comparatively suitable for the tick.

Patagonia and Chile represent two extremes of climatic types. Southern Chile, on its western mountainous strip of coastline, receives the moisture of the west winds and has therefore a very high rainfall throughout the year. Its vegetation type is dense forest and swamp. The result of this, however, is that eastwards, across Patagonia and as far as the coast, conditions are arid desert for most of the year. Only on the western seaboard, therefore, should conditions be suitable for the tick.

(*g*) *Known distribution*

The major regions outlined in the previous sections as being theoretically suitable for tick survival have been shaded on a world map (Fig. 5), and on the same map the various areas from which the tick has actually been recorded are indicated by dots. The American records (listed by Nuttall (1911, 1916), plus one in Florida, Giltner (1927)) must be accepted with reserve, as possibly including varieties and subspecies of *Ixodes ricinus*. The single record of this species from Panama (Dunn, 1923) is surprising, in view of the definite thermal limits to survival and activity of the tick. In the Old World, two tropical records occur, one in Eritrea (Franchini, 1927) and one in Hué (listed by Senevet & Rossi, 1926). These three records are inexplicable, and may possibly be due to faulty identification. The remaining Old World records fall within the specified limits of distribution, except for a few interesting exceptions. The eastern Mediterranean records, Macedonia and Smyrna (Senevet & Rossi, 1926), Palestine (Smith, 1930), and Libya (Zanon, 1923) are from areas definitely unfavourable as regards the major climatic conditions. They may possibly indicate limited foci of survival conditions, but this is improbable, except in the case of the Bengasi record (Libya). It is of interest, however, that three of the four, Smyrna, Palestine and Libya, are littoral regions.

The Armenian records (Yakimoff *et al.*, 1934; Lototzkii & Popov, 1934) are extremely interesting, in that the ticks occur in a limited area of definitely favourable conditions. The west winds, although parched in their passage over the European continent, renew their moisture over the Black Sea, and the Caucasus Mountains bordering it to the east thus receive a liberal supply of

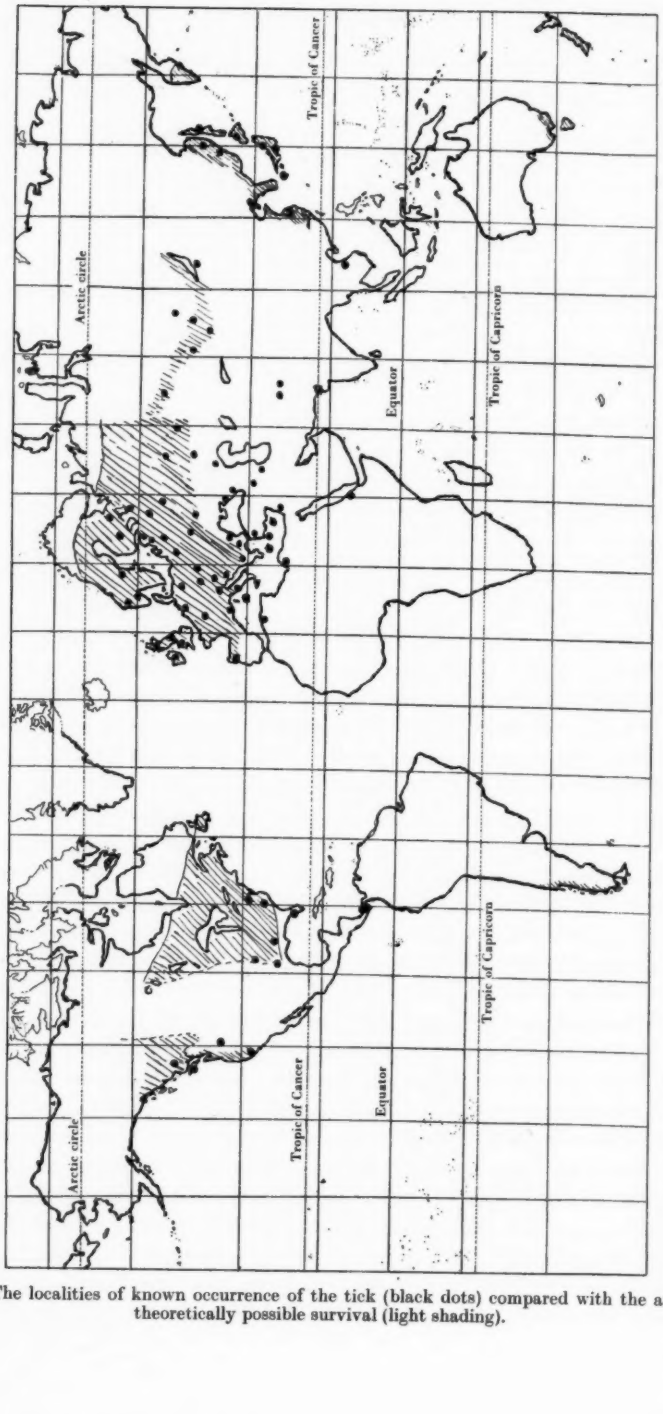

Fig. 5. The localities of known occurrence of the tick (black dots) compared with the areas of theoretically possible survival (light shading).

rain. The vegetation type consequently is dense forest. The second of these records is from a region at over 6500 ft.

Two records exist of occurrence of the species on the high plateau in the Pamir region, one in Chitral (Sachs, 1934) and one in Kashmir (Nuttall, 1916). The possibility of the occurrence of favourable areas of limited extent in this mountainous district has already been remarked on, since the region is outside the limits only because of scarcity of rainfall, the temperature requirements being satisfied. Locally advantageous conditions may also explain the Lake Baikal record (Olenev, 1929). Thus, the inhibiting factors in the Siberian region are considered to be periodic distribution of rain, absence of a protecting snow cover and absence of transition periods between the temperature extremes. Irkutsk, however, records 65 days with snowfall in the year, and, further, the extremes in the Lake Baikal area will be moderated appreciably, since lakes moderate the climate in their immediate vicinity to an extent roughly proportional to the size of the body of water (Miller, 1931). As regards the rainfall, seasonal absence of rain under such conditions of cool temperature does not necessarily indicate drought. As shown on Fig. 4, the moisture conditions remain sufficiently favourable throughout the year to support a woodland type of vegetation.

In the southern hemisphere, the only existing record of *Ixodes ricinus* is that of a single specimen in New Zealand (listed by Senevet & Rossi, 1926). Complete absence of the species from the antipodean region suitable to the tick would not have been surprising. Dispersal by ordinary means could hardly result in its introduction, since the longest period the tick remains attached to a host is a matter of 2 weeks, and the tick would require to traverse the tropical and equatorial unfavourable belt to reach the antipodes by mechanical carriage in the active, unfed state. The single record is, therefore, all the more surprising, since the suitability of the climate would indicate a high density of population if the tick were present.

The difficulty of surviving the migratory passage from the northern hemisphere readily accounts also for the apparent absence of the tick from the limited favourable area on the western seaboard of Chile, unless, as seems improbable, it were already established in southern America before the establishment of the present climatic belts.

VI. SUMMARY

The biotic factors in the environment of the tick, *Ixodes ricinus*, exercise little effect on the geographical or local distribution of the species, or on its seasonal prevalence.

The local distribution is determined by edaphic factors, a wet, mossy, or peat soil and a dense mat of old vegetation or a rank growth being necessary for survival of the tick. In Britain, the critical season for survival is summer, during which the moisture factor in the microclimate acts in a limiting capacity.

The seasonal prevalence is determined by temperature. Within limits, which appear to correspond to air-temperature limits of 7 and 16° C. (weekly maxima), the unfed tick climbs the vegetation, and thus readily obtains a host. In Britain, it is inactivated in winter by the cold, and in summer it is less readily picked up by hosts because of its positive geotropic response to the stimulus of high temperatures.

The summer is, in Britain, the optimum season for development, which also proceeds to some extent in winter. Autumn and spring are parasitisation seasons. The life cycle, involving a parasitisation and a development season for each stage, requires a minimum of $1\frac{1}{2}$ years. The period may extend to $4\frac{1}{2}$ years.

The possible world distribution of the species is limited primarily by temperature. Thus, microclimatic extremes of −14 and 35° C. limit the range through which the tick can survive. A period of at least 3 months with the mean air temperatures over 10° C. is necessary for development, while the mean air temperature of the coldest month must not exceed 10° C. to allow of parasitisation occurring.

Within the areas delimited in relation to the temperature requirements of the tick, distribution is governed by the moisture factor. An index of the suitability of an area within the temperature limits is afforded by the type of vegetation; forest and woodland, including grass and cultivation areas, as opposed to prairie and steppe, indicate suitable moisture conditions.

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STUDIES IN TICK-BORNE FEVER OF SHEEP

II. EXPERIMENTS ON TRANSMISSION AND DISTRIBUTION OF THE DISEASE

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I. Experiments on transmission

(i) *Introduction*

In a previous paper (MacLeod & Gordon, 1933) it was shown that tick-borne fever is transmitted by the female and the nymph of *Ixodes ricinus*; the larva appeared to be non-infective. The present paper describes experiments bearing on the history of the infective agent in the tick, and experiments on the transmission of the disease by emulsions of ticks. Finally, experimental evidence is given of the wide distribution of the disease on Scottish hill pastures.

A complete study of the life history of the infective agent in its tick host would involve histological examination of ticks at different periods after infection, and has not so far been attempted. The following observations are confined to those aspects of the history which have a practical bearing on the problem of control of the disease. Thus, (1) it is important to know whether the infective agent can pass through the egg stage of the tick, *i.e.* whether a female which has fed on infected blood can transmit the infection to its progeny, which may number 2000, or even more; (2) whether an infective tick, after inducing the disease in a sheep, can reinfect itself from that same animal.

It would appear that even if tick-infected pastures are kept free from sheep for a period of 3 years a certain minimum population of ticks can

maintain itself on the alternative hosts represented by vermin and birds (MacLeod, 1934). The success of starvation measures directed against the tick will therefore depend on the possibility of the infective agent failing to survive in this minimum population, rather than on the eradication of the tick itself. It thus becomes necessary to know:

(3) How long an unfed tick remains infective, and

(4) Whether infective ticks which feed on non-susceptible animals cleanse themselves of infection, or retain their infectivity.

(5) Finally, it is important, from the point of view of control measures involving dipping, to know the minimum interval between attachment of the tick and transference of the infective agent from the tick to the sheep.

(ii) *Absence of hereditary infection*

The previous paper recorded several unsuccessful attempts to demonstrate transmission of the infective agent from female to larva, and the conclusion was reached that the larval stage was not infective. Further experiments with larvae, bred from females which had engorged on affected sheep, confirmed this finding. There remained the possibility that infection may pass through the egg, remain latent in the larva, and appear in the nymphal stage. Regendanz & Reichenow (1932) suggest that this occurs in the case of *Rhip. sanguineus*, the vector of *Piroplasma canis*. To test the possibility, the following experiments were carried out.

Tups **451, 453,** *and* **499.** Gorged females were collected from a naturally occurring case of tick-borne fever (sheep 169—Gordon *et al.* 1932), and the resulting larvae were fed on a sheep without producing a reaction. The gorged larvae were bred to nymphs, and these were infested when 2 months old on two tups, Nos. 451 and 453. Neither tup reacted. Five months later, others of the same lot of nymphs were fed on tup 499. The sheep did not react.

Since neither the larval nor nymphal progeny of females which presumably acquired infection were themselves infective, it seems highly probable that the egg stage of the tick is an effective barrier to the transmission of the tick-borne fever virus from one generation of the tick to the next.

(iii) *Limits of infective period in unfed ticks*

The results of earlier experiments on the transmission of tick-borne fever suggested that the moulted tick becomes infective only after the lapse of a definite period of time. The experiments may be summarised as follows.

Stockman (1918, 1919) fed nymphs on a case of so-called louping-ill, which was probably a case of tick-borne fever. The adults, infested on sheep (*a*) immediately after moulting, and (*b*) 15 days after moulting, produced no reaction, whereas those infested on a sheep 58 days after moulting caused a temperature reaction.

In the writer's experiments, a sheep (tup 500) was infested with 2–4 weeks old nymphs and did not react, whereas others of the same lot of nymphs, when 4–6 weeks old, produced a reaction in this sheep.

Later experiments, however, in which ticks of definitely known age were used, disproved the possibility of an incubation period in the moulted tick, *e.g.* tup 654 was infested with nymphs 7–12 days old, tup 614 with nymphs 12–19 days old, and tup 615 with nymphs 23–28 days old. All three sheep reacted.

The newly moulted tick requires about a week to harden; it follows that ticks are infective as soon after moulting as they are able to attach.

Since the unfed tick can survive for over a year while awaiting a host, it is of interest to know whether the contained infective agent is viable equally with its invertebrate host. The point is of more than academic importance, for it has an important bearing on control measures. It was already known (see MacLeod & Gordon, 1933) that nymphs could retain their infectivity for at least 9 months.

Tup 492. Gorged nymphs were collected from naturally occurring cases of the disease, bred to adults, and stored at room temperature. Thirteen to fourteen months after moulting, they were infested on tup 492. Some of this field material must have been collected from a sheep undergoing a latent attack of louping-ill, for there developed in tup 492 a typical diphasic febrile reaction, beginning on the second day after infestation and ceasing by the ninth day. Two days later, *i.e.* on the eleventh day, another reaction developed. The reaction was mild, the temperature exceeding 106° F. on 1 day only, and returning to normal in about 8 days. Blood drawn from tup 492 during this second reaction was inoculated into three normal sheep, and produced tick-borne fever reactions.

The slight nature of the tick-borne fever reaction, and the long incubation period, suggested the possibility that the infective agent had become attenuated. That its viability may not be so great as that of the tick itself is further suggested by the following experiments.

Tup 618. Some females of the 1931 field material (see MacLeod & Gordon, 1933) were infested when 18 months old on tup 618. No reaction occurred.

Tup 465. Larvae infected on tup 903 during its reaction to tick-borne fever, and bred to nymphs, were infested 18 months later on tup 465, which did not react.

Tup 900. Larvae were infected on a reacting tup, No. 288, and bred to nymphs. Fifteen to sixteen months later they were infested on tup 900, without producing a reaction.

The evidence, although inconclusive, suggests that the tick-borne fever virus dies out in the unfed tick after a period of about 14 months.

(iv) *Influence of a blood meal on subsequent infectivity*

In nature, after a tick has acquired infection from a sheep, and has moulted, it does not necessarily follow that its next host will be an animal susceptible to infection. The following possible sequences, and the influence of each on the subsequent pathogenic potentiality of the tick, require to be considered:

(1) The infective tick may feed on a susceptible animal, which reacts to the infection before the tick has completed engorgement. In this case the tick may renew its infection from the infected blood ("perpetuation of infection"), or, possibly, its tissues may have so reacted to the previous presence of the virus that renewed infection does not occur.

(2) The infective tick may feed on a susceptible animal and detach itself before the host reacts, in which case the tick may either have cleansed itself of infection, or, alternatively, have retained a certain amount of "residual infectivity", as in the case of *Dermacentor reticulatus* transmitting equine piroplasmosis (Du Toit, 1919).

(3) The tick may feed on a non-susceptible animal, either a naturally refractory animal, or a sheep or other normally susceptible animal which has acquired immunity by virtue of a previous infection. In the event of the tick feeding on a refractory animal, it may either cleanse itself, or the virus may remain latent, and appear in the next stage of the tick. "Latent survival" of this type has been shown to occur in the transmission of piroplasmosis of cattle (Sergent *et al.*, 1931). The effect on the tick of engorgement on an immune sheep could not be explored, since it is difficult to induce in a sheep complete immunity to tick-borne fever; further, in sheep which are relatively immune by reason of a previous attack, the blood remains infective for long periods after recovery.

(*a*) *Perpetuation of infection.*

The term "perpetuation of infection" is, strictly speaking, not applicable in the case of tick-borne fever, since the infection is not transmissible from mother to offspring, so, at most, the infection could only be perpetuated within one generation, *i.e.* from nymph to female. It is used here for convenience, however, to distinguish the automatic renewing of infection from the apparent survival of original infection—the "residual infection" next discussed.

One instance will be sufficient to illustrate the sequence.

Tup 588. Infected nymphs produced a reaction in tup 456, the reaction beginning on the 7th day after infestation. The nymphs were collected on the 6th and 7th day, *i.e.* the sheep had reacted before all the ticks had completed engorgement. They were bred to adults and the females allowed to attach on tup 588, which developed a tick-borne fever reaction 12 days later.

(*b*) *Residual infectivity, Tups* 582, 891, 902, 962, *and* 964.

In five instances where a reaction was produced by nymphs, these were collected before the reaction began, bred to adults, and the females subsequently tested for infectivity. The experiments and their results are summarised in Table I.

Table I

Host sheep	Incubation period in days	Nymphs recovered	Females infested on sheep	Result
286	6	5th and 6th day	582	T.B.F.
653, 656	6	5th and 6th day	891	No reaction
659	6	5th day	902	T.B.F.
903	7	4th and 6th day	962	T.B.F.
885	5	5th day	964	T.B.F.

T.B.F. = tick-borne fever.

Thus in four out of five cases, infective nymphs which had produced reactions in sheep, and had detached themselves before the reactions became manifest, were capable as adult females of infecting sheep, *i.e.* had retained a "residual infectivity".

This result is open to the objection that, although the reaction had not begun when the gorged ticks detached themselves, the blood stream of the sheep may have contained a sufficiently high concentration of virus to infect the ticks, and an apparent residual infectivity in the ticks may have been due in reality to reinfection. This objection, however, is inapplicable in the case of nymphs which engorge on insusceptible animals, and, as will be shown later, such nymphs are still capable as adult females of producing infection. It appears, therefore, that infective ticks feeding on susceptible hosts and detaching themselves before these hosts react, do not cleanse themselves of infection, but retain their infectivity.

(*c*) *Latent survival of infection.*

Many ticks in nature find their hosts at one stage or another among vermin and birds, and it is necessary therefore to determine whether infection in ticks will survive such a blood meal and the subsequent moult. Neither the rabbit nor the guinea-pig appear to be susceptible to tick-borne fever, and these animals, being convenient for experiment, have been used to represent hosts naturally refractory to the disease.

Tups 887, 888, 440, 443, *and ewe* 847. Individuals of an infective strain of nymphs were fed on a guinea-pig and bred to adults. Four of these were infested on sheep 847. No temperature reaction developed in this sheep, but on the 14th day it died from causes unconnected with tick-borne fever. Further individuals of this guinea-pig strain of females were infested on tups 887 and 888. Neither reacted.

A strain of infective nymphs was prepared, and their infectivity proved by infesting some on tup 954, which reacted. The nymphs were allowed to engorge on a guinea-pig and a rabbit, collected separately, and bred to adults. The females from the guinea-pig were then infested on tup 440, those from the rabbit on tup 443. The sheep developed tick-borne fever on the 9th and 5th day respectively.

(v) *Time of occurrence of infection in sheep*

It has been shown by Cowdry & Danks (1933) that the process of attachment of ticks (*Rhip. appendiculatus*) does not necessarily involve an immediate penetration of the mouth-parts into a blood vessel. There occurs a general lysis of the tissue in which the mouth-parts are imbedded, due to the action of a salivary cytolysin. This lysis involves the walls of minute blood vessels in the neighbourhood, and the tick ingests the extravasated blood, with tissue cells and lymph. Further, it is known that the salivary glands of *Ixodes ricinus* are activated by the blood first ingested. Corresponding to this, there is an increase in the rate of ingestion of the blood meal, engorgement proceeding most rapidly during the last days of attachment. It is conceivable, therefore, that an infective agent in the salivary glands of the tick may not pass to any

great extent into the blood stream of the host for some time after attachment is effected. Such a time lag between attachment and infection would assume considerable significance in the event of a serial dipping of stock being instituted to control an outbreak of the disease; the maximum interval between the dippings would in such case be equal to the sum of the time lag and the period of protection from infestation conferred by the dip.

Tups 885, 886, 892, 894, 895, 896, *and* 897. Seven tups of the same age, breed, and history were each infested with 20 nymphs of a known infective strain. Tups 886 and 896 were thoroughly dipped in an arsenical bath after 24 hours, tups 892 and 894 after 48 hours, tups 895 and 897 after 72 hours, and tup 885 was left as a control. Table II summarises the experiment and results.

Table II

Sheep	History	Result
886	Ticks attached for 0–1 day	No reaction
896		No reaction
894	,, 1–2 days	No reaction
892		Reaction on 8th day
895	,, 2–3 days	Reacted on 6th day
897		Reacted on 6th day
885	,, 4–5 days	Reacted on 6th day

It would appear from this experiment that infection with tick-borne fever occurs between the 1st and 2nd day after attachment of infective nymphs, *i.e.* that there is a time lag between attachment and infection of at least 1 day.

(vi) *Transmission by emulsions of infective ticks*

The previous paper (1933) described a limited number of experiments in which the authors failed to transmit the disease by subcutaneous injection of saline emulsions of ticks. These experiments were extended, the tick material being injected (1) untreated, (2) after incubation at different temperatures, and (3) after partial engorgement of the ticks in order to activate the salivary glands. The results were consistently negative, despite the fact that in several instances the strains of ticks from which material for injection was taken were shown by simultaneous check experiments to be readily infective when infested on sheep. The experiments are summarised in Table III.

The conditions of the experiments which gave these negative results appeared to be comprehensive, and so an explanation was sought in the factor common to them all—the preparation of the emulsions. In all the above experiments the ticks had been emulsified in physiological saline; this was now replaced by serum obtained from the sheep to be inoculated.

Tups 432, 433, 435, *and* 463. Tup 433 was infested with nymphs of a known infective strain, and reacted. Twenty-five others of the same strain were emulsified in serum and inoculated subcutaneously into tup 432. This tup did not react. On the day following infestation of 433, 12 of the attached nymphs were removed, emulsified in serum, and inoculated into tup 435. On the 2nd day after infestation another 12 were removed, emulsified in serum, and inoculated into tup 463. These two tups developed tick-borne fever on the 6th and 7th day respectively.

Tups 437 *and* 468. The above experiment was partially repeated in that 10 nymphs of the same strain were emulsified in serum and inoculated into tup 465, 10 others being infested on tup 437 as a control. Tup 437 reacted, whereas 465 did not react.

Table III

Group	Sheep	History of inoculum	Infectivity control	Result	Remarks
I. Untreated	653	20 nymphs, 614 strain	659 infested same day, and reacted	Negative	653 subsequently shown to be susceptible
	433	25 nymphs, 956 strain	961 infested, and reacted	Negative	—
II. Incubated	310	17 adults (field material) incubated 24 hours at 30° C.	—	Negative	310 afterwards susceptible
	407	73 adults (field material) incubated 24 hours at 25° C.	—	Negative	—
	656	20 nymphs, 614 strain, incubated 24 hours at 27° C.	As for 653	Negative	656 afterwards susceptible
III. Partially fed:					
(*a*) 2 days	603	10 nymphs, 614 strain, fed for 2 days on 653	653 reacted. Some of recovered ticks shown to be still infective as females	Negative	603 afterwards susceptible
(*b*) 3 days	415	7 females, field material, fed for 3 days on 244	244 reacted	Negative	—
	657	10 nymphs, 614 strain, fed for 3 days on 659	659 reacted. Some of recovered ticks shown to be still infective as females	Negative	657 afterwards susceptible
(*c*) 4 days	607	10 nymphs, 614 strain, fed for 4 days on 656	656 reacted. Some of recovered ticks shown to be still infective as females	Negative	—
IV. Gorged	624	12 nymphs, 614 strain, recovered from 659 before reaction began. Incubated 5 days at 27° C.	As for 657	Negative	—
	608	10 nymphs, 614 strain, recovered from 653 before reaction began. Incubated for 4 days at 25° C.	As for 603	Negative	—
	579	10 nymphs, 614 strain, recovered from 656 before reaction began. Incubated 4 days at 25° C.	As for 607	Negative	—

That the positive results obtained were due to the absence of saline, and not to some other departure from the earlier procedure, is shown by the following experiment.

Tups 457, 449, *and* 465. Tup 457 was infested with infective females. On the 2nd day of engorgement, 22 of these were removed and emulsified in 2 c.c. of serum. The emulsion was diluted with 2 c.c. of saline, and inoculated into tups 449 and 465. Tup 465 did not react, and tup 449 reacted to tick-borne fever infection after an incubation period of 18 days.

These results suggest that the negative nature of the earlier results was due to the action of the saline on the infective agent in the tick tissues. When sheep serum is used in the preparation of the emulsion, tick-borne fever can

be induced by inoculation of sheep with emulsified ticks. Activation of the virus by allowing the ticks to commence a blood meal would appear to be a desirable, if not a necessary, preliminary treatment.

(vii) *Summary*

(1) Female *Ixodes ricinus* ticks which feed on a sheep infected with tick-borne fever are not capable of transmitting the infective agent to their progeny. Only the nymphs and females, therefore, can be infective to sheep. In the case of an infective female, the infection may have been acquired, (*a*) in the immediately preceding stage, or (*b*) in the larval stage, the infective agent remaining in the tissues of the tick during the nymphal engorgement and subsequent moult. This appears to occur irrespective of whether the host on which the nymph feeds is a susceptible or an insusceptible animal.

(2) Ticks which have acquired infection are infective as soon after moulting to the next stage as they are able to attach to hosts. Unfed ticks were not found to be infective after a period of about 14 months.

(3) Infection is transmitted to the sheep in the course of the 2nd day after attachment of infective nymphs.

(4) The disease may be transmitted to sheep by inoculation of emulsions of infective ticks, if these have been allowed to commence engorgement. The use of saline for such emulsions appears to be inadvisable.

II. Distribution of the disease

Since the disease has only recently been recognised as a distinct entity, it is difficult to find in the literature references which may be taken with any confidence to indicate tick-borne fever. It is highly probable that the febrile reactions in sheep described by Stockman (1918, 1919) were in many instances those of tick-borne fever. These reactions were produced in sheep by infestation with ticks, or by inoculation of sheep tissues, obtained mainly from "infected" farms in Northumberland.

Walton (1927) records a disease of lambs of undetermined origin in North Wales, associated with tick infestation. He mentions a general loss of condition in the sheep flocks during the tick season, and a high mortality among the lambs from pyaemia due to mixed infection. Surviving lambs showed a marked deterioration. The frequent occurrence of pyaemic infection among the lambs is a common observation on tick-borne fever infected farms, while a characteristic of the disease, as it occurs naturally, is the general deterioration in condition of the infected flock. It is very probable that the disease condition encountered by Walton was due primarily to tick-borne fever.

The fact that the disease is intimately associated with louping-ill would lead one to expect that its distribution would extend to the limits of distribution of the latter. It had been experimentally produced only with material obtained from the Ettrick valley; an effort was therefore made roughly to

determine its distribution in Scotland. The vector, *Ixodes ricinus*, is confined in its Scottish distribution to the Border uplands, the central Grampians, Argyll, and the hilly country of northern, and particularly north-western, Scotland (MacLeod, 1932). During the tick seasons of 1932 and 1933, gorged larvae and nymphs were collected from the native stock in certain of these districts, and tested for the presence of infection.

Newcastleton district: tups 890 *and* 903. Gorged larvae and nymphs were collected in May, 1933, from adult sheep on a hill farm in the Newcastleton district, at the head of the Solway Firth; a collection of larvae was made from lambs, about a month old, on the same farm. These ticks were bred to the next stage, and the ewe collection infested on tup 890, the lamb collection on tup 903. Both sheep reacted.

South Ayrshire: tup 655. Gorged nymphs collected in May, 1932, from native stock on a hill farm near Girvan were infested as adults on tup 655, which developed a tick-borne fever reaction.

Argyllshire: tups 652, 889, *and* 898. Gorged nymphs collected in 1932 near Oban failed to produce a reaction, as adults, on tup 652. A further collection of nymphs and larvae was made in 1933. The females resulting from the nymphs failed to produce a reaction in sheep 889, but the nymphs resulting from the larvae produced a tick-borne fever reaction in tup 898.

Central Perthshire: tups 653 *and* 892. Gorged nymphs and larvae, collected in 1932 on a hill farm in Balquhidder, were infested in their next stage on tup 653. No reaction developed. In 1933, four gorged nymphs obtained from a moribund lamb in the Trossachs yielded 2 females, which were infested on tup 892 without result.

Dunkeld district: tup 901. The country round Dunkeld, and in the eastern Grampians generally, is more fertile than is the country further west, the soil being generally less acid, and producing a better quality of herbage. The hill sheep are usually in good condition, and comparatively free from disease. It was not expected that the ticks in this area would be infected. In 1933 a collection of gorged nymphs was made from the stock of a hill farm near Dunkeld. The resulting females were infested on tup 901, which developed tick-borne fever.

In an investigation of this type, where the ticks are collected at random from apparently healthy sheep, negative results cannot be regarded as significant, when only small numbers of experiments are concerned. The failure, in two attempts, to demonstrate the presence of tick-borne fever in Perthshire does not indicate that the disease is not present there. The presence of the virus has frequently been demonstrated in the blood of ailing sheep from the Balquhidder district (W. S. Gordon, private communication). The evidence of stockmasters and shepherds compels the belief that the disease is indeed prevalent in this area.

The fact that its presence has been demonstrated in three areas in the Grampians—Argyll, central Perthshire, and Dunkeld—and in three widely separated points in the south of Scotland—Ettrick, Newcastleton, and south Ayrshire—suggests the probability that the disease is, in fact, prevalent over all the hill country of Scotland where ticks exist.

Experimental evidence from the Ettrick valley investigations revealed that practically all the ticks on an infected farm harboured infection: the fact that gorged larvae collected from young lambs in the Newcastleton district

were infective as nymphs further supports this finding, since these lambs, although only a month old, had already reacted to infection, and were in turn infecting their attached ticks.

If ticks are present on sheep in appreciable numbers, if there is a high general mortality among the stock, death being due apparently to a variety of disease factors, and especially if the lambs exhibit a general falling off in health and condition when a few weeks old, the period of ill-health lasting from 2 to 3 weeks, then it is a reasonably safe assumption that tick-borne fever is actively present, and is exacting its toll of health and life.

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ON A COLLECTION OF PARAMPHISTOMIDAE FROM MALAYA, WITH REVISION OF THE GENERA *PARAMPHISTOMUM* FISCHOEDER, 1901, AND *GASTROTHYLAX* POIRIER, 1883

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(With 7 Figures in the Text)

Introduction

There is still some difference of opinion as to the classification of what Rudolphi in 1801 called the Amphistomata. Fischoeder (1901–4) regarded all "Amphistomes" as belonging to a single family, the Paramphistomidae. Stiles & Goldberger (1910) proposed the elevation of the group to the status of a superfamily, the Paramphistomoidea, comprising three families, Paramphistomidae, Gastrothylacidae and Gastrodiscidae. Braun (1911), Odhner (1911), Looss (1912) and Stunkard (1925) have maintained Fischoeder's view; Maplestone (1923) and Travassos (1934) have preferred that of Stiles and Goldberger.

In his comprehensive classification of Trematoda, Poche (1926) erected the "supersuperfamily" Paramphistomida for three families, Paramphistomidae, Dissotrematidae and Angiodictyidae. This entails a rejection of the superfamily of Stiles & Goldberger, which corresponds to the first-mentioned of Poche's (1926) families. Fukui (1929), who does not mention the work of Poche, regards the families Paramphistomidae, Angiodictyidae and Opistholebetidae as being of equal rank with acknowledged families of Distomata and uses the term "Amphistome" for convenience in denoting the group.

Materials

The collection comprises 30 sets of specimens, altogether approximately 2500, collected in Malaya by Mr G. B. Purvis, F.R.C.V.S., when District Government Veterinary Surgeon. The writer extends his thanks to Dr H. A. Baylis, of the British Museum (Natural History), for allowing him to examine the collection and for assistance in the matter of obtaining literature. In a letter to the writer, Mr Purvis states that material of doubtful location in the body of the host was consistently rejected in the interests of accuracy. 16 of the sets of specimens were taken from various situations in the bodies of the ox, cow, calf and "Siam bull" (all *Bos indicus*, the zebu); 7 sets came from the body of the domestic goat (*Capra hircus*); and 7 sets were obtained from the

common Asiatic buffalo (*Bos bubalus*). Most of the specimens were well preserved and suitable for general histological study in serial sections, but a few were suitable for study only as whole mounts. Paracarmine was used as a general stain, with good results, and routine clearing in clove oil was found to be quite satisfactory. It might be mentioned that all specimens mounted whole were examined carefully while still in the clearing fluid, the general method being to roll the specimen over and over repeatedly until it had been viewed from all possible aspects. This method is indispensable if a true picture of the contours of organs like the testes is desired.

LIST OF SPECIES ARRANGED SYSTEMATICALLY

Family PARAMPHISTOMIDAE Fischoeder, 1901

Subfamily GASTROTHYLACINAE Stiles & Goldberger, 1910

Genus **Gastrothylax** Poirier, 1883

G. elongatus Poirier, 1883
G. cobboldii Poirier, 1883
G. crumenifer (Creplin, 1847)
G. spatiosus Brandes, 1898

Subfamily PARAMPHISTOMINAE Fischoeder, 1901

Genus **Paramphistomum** Fischoeder, 1901

P. cervi (Schrank, 1790)
P. explanatum (Creplin, 1849)
P. siamense Stiles & Goldberger
P. cotylophorum Fischoeder, 1901
P. gotoi Fukui, 1922
P. orthocoelium Fischoeder, 1901

NOTES ON THE GENUS *GASTROTHYLAX* POIRIER

In erecting the family Gastrothylacidae, Stiles & Goldberger (1910) claimed recognition for four distinct genera, *Gastrothylax*, *Fischoederius*, *Carmyerius* and *Wellmanius*, of which the last three were new. With apparent justification, Maplestone (1923) disposed of the last-named genus on the ground that its only species, *Wellmanius wellmani*, is identical with *Carmyerius spatiosus*. In the writer's opinion the remaining three genera must be reassembled into the original one, *Gastrothylax*, the structural differences between forms referred to these genera being of insufficient importance to warrant generic separation. This is in accordance with the opinion of Fukui (1929) who disagrees with the writer in regarding the differences as being of subgeneric value.

The genus *Fischoederius* was erected for 5 species, *elongatus*, *cobboldii*, *fischoederi*, *siamensis* and *ceylonensis*, of which only the first two are of undoubted validity. Of the two species of the genus *Gastrothylax*, *crumenifer* and *compressus*, only the former now stands. In both instances the synonymy is accepted by Fukui (1929) and Travassos (1934) and also by the writer. Further, Maplestone has reduced the number of valid species of *Carmyerius* by synonymy from 8 to 4, *spatiosus*, *wenyoni*, *cruciformis* and *gregarius*, to which his species *exoporus* must be added. To Maplestone's five synonyms of *C. spatiosus*,

namely, *synethes*, *minutus*, *mancupatus*, *bubalis* and *wellmani*, Fukui would add a sixth, *cruciformis*, which the former author could distinguish from *spatiosus* only by its very small size in the gravid condition. Stunkard (1925) would apparently prefer to regard *wenyoni* as yet another synonym of *spatiosus*, but the union of the excretory canal with Laurer's canal to open by a common pore appears to be a feature of specific distinction.

What remains, therefore, of the species recognised by Stiles & Goldberger under 4 generic titles is a set of 7 species which may be considered genuine, *elongatus*, *cobboldii*, *crumenifer*, *spatiosus*, *gregarius*, *exoporus* and *wenyoni*. The arrangement of these species in the three genera adopted by Maplestone is based upon two characters taken either singly or together: the relative positions of the testes and the slightly variable course of the uterus. The testes may lie side by side or show an obliquely dorso-ventral arrangement, and the uterus may pursue its entire course approximately in the sagittal plane, or may run on opposite sides of this plane in anterior and posterior halves of the body, crossing it about the middle of the body. The former character is more important than the latter, but neither by itself is considered to be of generic value.

In the opinion of the writer, *Gastrothylax spatiosus* must be separated from the five forms Maplestone deemed identical with it, and these are grouped under the specific title *G. synethes* Fischoeder, 1901. The 8 species of the genus *Gastrothylax* recognised by the writer may be separated by the following key:

KEY TO SPECIES OF *GASTROTHYLAX*

1. Genital pore opens into the ventral pouch 2
   Genital pore opens outside the ventral pouch ... ... **exoporus**

2. Excretory canal and Laurer's canal with separate openings 3
   Excretory canal and Laurer's canal share a common opening ... ... **wenyoni**

3. Uterus confined to the sagittal plane 4
   Uterus crosses from one side of the sagittal plane to the other near the middle of the body **crumenifer**

4. Testes side by side 5
   Testes obliquely dorsal and ventral or in tandem 6

5. Diameter of the posterior sucker less than one-tenth of the length of the body **elongatus**
   Diameter of the posterior sucker greater than one-tenth of the length of the body **cobboldii**

6. Caeca do not reach back to the level of the testes ... ... **gregarius**
   Caeca extend back to the level of the testes 7

7. Ventral pouch circular or pentagonal in transverse section ... ... **spatiosus**
   Ventral pouch triangular in transverse section ... ... **synethes**

Gastrothylax elongatus Poirier, 1883

Synonyms: *Fischoederius fischoederi* Stiles & Goldberger, 1910
F. siamensis Stiles & Goldberger, 1910
F. ceylonensis Stiles & Goldberger, 1910

Since its discovery in the stomach of a Javanese gayal, this species has become a well-known paramphistomid of cattle in the Far East (Ceylon, Siam, Java and Celebes). Fukui (1929) found it to be common and widespread in Japan and Formosa, and Tubangui (1933) has recorded its occurrence in the Philippines. In view of its abundance in the present collection, it must be regarded as a very prevalent paramphistomid in Malaya, where it occurs in all three hosts examined. Neither *Bos indicus* nor *Capra hircus* is mentioned in the host list of Travassos (1934), and the latter is recorded for the first time.

Abundant material was available for study, namely:

6 sets (110–150 specimens each) from oesophageal groove of cow (sets 2, 5, 6, 8, 9 and 10).

3 sets (more than 300 specimens in all) from rumen of ox (3, 13, 14).

2 specimens from oesophagus of buffalo (29).

3 sets (more than 180 specimens in all) from rumen of buffalo (17, 20, 21).

1 set (nearly 600 specimens) from rumen of goat (1).

In hosts other than the goat, *Gastrothylax elongatus* was found in association with other paramphistomids, and in dominant numbers. In the cow, it was found with *G. cobboldii* in proportions varying from 7/2 to 15/2, and in the mean proportion of 5–6/1; in the ox, with *Paramphistomum cotylophorum* in the approximate proportion of 10/1; and in the buffalo, with *Gastrothylax spatiosus* and *G. crumenifer* in significant predominance. In the goat, this species was found in isolation, but in a similar situation within the body of a second goat *Paramphistomum cotylophorum* was found, suggesting the possibility of association as met with in the ox.

The synonymy has been discussed at some length by Maplestone, has been accepted by Fukui and by Travassos, and does not call for any comments.

According to Fischoeder (1903) the range in size of this species is 10–20 mm., specimens being mostly 13–16 mm. long. Fukui (1929) obtained from Formosa more than 200 specimens 1–7 mm. long and mostly 3–4 mm. long. Even the smallest specimens had eggs in the uterus. With due regard for this condition and also for minor differences from specimens of the more usual size (a relatively larger oral sucker, longer intestinal caeca and a spindle-shaped pars prostatica), Fukui referred his minute specimens to a new variety, *japonica*.

The writer's specimens from the ox vary over the wide range of 8–24 mm., and the very smallest are immature. Specimens from the goat are much smaller (4–14 mm. long), and only the largest are mature. In essential structural details the specimens conform quite closely with Fischoeder's descriptions and figures, though the larger specimens appear to be slenderer than the smaller

ones. Having in mind Maplestone's observations on the variable length of the caeca, the writer made a few measurements by which the sizes of certain organs in large and small well-extended specimens may be compared. The results set out in Table I (mean measurements of six) show that the proportions of these organs are not identical in large and small individuals and indicate that changes in form take place during growth in size. Small individuals have a relatively wider body than large individuals, and also relatively larger testes, a longer and wider oral sucker and a longer alimentary canal, *but* a shorter and narrower posterior sucker.

Table I

	(*a*) Large individuals mm.	(*b*) Small individuals mm.	Ratio (*a*)/(*b*)
Body length	19·4	9·7	2·0
Maximum width	2·9	1·9	1·5
Oral sucker (length)	0·9	0·5	1·8
Oral sucker (width)	0·7	0·45	1·5
Posterior sucker (length)	1·35	0·6	2·25
Posterior sucker (width)	2·1	0·9	2·3
Length of entire gut	7·5	4·9	1·5
Anterior testis	1·6 × 1·1	0·85 × 0·65	1·9...1·7
Posterior testis	1·25 dia.	0·85 × 0·75	1·5...1·7

The small specimens of Fukui possess characters which he considers worthy of a separate variety, yet in the larger oral sucker and longer gut they merely show a peculiarity of growth which is correlated with small absolute size. During growth the oral sucker apparently becomes progressively relatively smaller, the gut relatively shorter and the posterior sucker relatively larger. Growth would appear to be of a graded nature with a region of high intensity in the posterior part of the body. In such organisms as show this type of growth absolute size is a valuable criterion and it must not be disregarded in descriptions of Paramphistomidae.

Another important feature of *Gastrothylax elongatus* is variability in the positions of the testes. In well-extended specimens of large size, the dorsal testis is generally slightly anterior to the ventral testis (Fig. 1 A), and the ovary lies between it and the excretory vesicle. In some specimens with large testes the dorso-ventral arrangement gives place to a condition in which the dorsal testis may be almost its own diameter in front of the ventral testis, and in a number of large specimens examined by the writer the testes lies definitely one behind the other. When the testes attain their maximum size, there is scarcely the space available for their accommodation in the dorso-ventral positions, and this is the apparent cause of the almost true tandem positions. Even in such specimens, however, there is little likelihood of taxonomic difficulties arising, because the organs invariably lie in the sagittal plane, and this fact, taken with the shortness of the caeca, provide the specific characters.

Gastrothylax cobboldii Poirier, 1883

Maplestone lacked specimens of this undoubtedly genuine Asiatic species, which Fukui states is rare in Japan. More than 150 specimens were studied by the writer, 15–40 in each of 6 sets of *G. elongatus* from the oesophageal groove of the cow, and a fraction of 180 specimens (of which the remainder were *G. crumenifer*, *G. spatiosus* and *Paramphistomum gotoi*) from the rumen of the buffalo. The specimens are 7–16 mm. long and thus show a greater range in size than Fischoeder's, which were 8–10 mm. long.

According to Fischoeder, the extent of the caeca to a point behind the testes and thus near the hinder end of the body is the important character distinguishing this species from *Gastrothylax elongatus*. Maplestone accepts Fischoeder's ruling but points to the fact of variability in length of the caeca in all members of the group. The writer would insist, however, that even in unstained and uncleared specimens there is no difficulty in distinguishing between the two species, because *G. cobboldii* is relatively shorter, thicker and more heavily built and also more sharply truncated posteriorly. The body is slightly constricted just in front of the posterior sucker, which, because of its relatively large size, gives rise to a prominent bulge at the posterior end of the body (Fig. 1 B). In specimens 10–11 mm. long the ratio of the width of this sucker to the length of the body is about 1/4·7 as against 1/19 in slightly smaller specimens of *G. elongatus*. Fischoeder states that the greatest measurement of the sucker is about one-quarter of the length of the body, a fact which has not been given its due prominence in the literature. The ratio of 1/10 by which *G. cobboldii* and *G. elongatus* are separated in the writer's key thus allows a considerable margin for individual variations and emphasises more general differences between the species than are seen in the variable lengths of the caeca.

The caeca differ from those of *G. elongatus*, however, and provide an important subsidiary taxonomic character. They are wider and consistently very much longer, reaching almost if not quite to the level of the posterior sucker. They pass along the body in a series of waves which take up at least one-fifth of the width of the body and run dorso-ventrally as well as from side to side. In some specimens the caeca are constricted at indeterminate points, intervening regions being distended, a character due, perhaps, to local contractions perpetuated in fixation. In not a single instance were the peculiar twists visible as shown in Fischoeder's Fig. 63.

The writer's specimens have small testes, which are ovoid, have their long axes transverse, and are divided into a number of fine lobes. They lie consistently in the sagittal plane, one slightly dorsal and anterior to the other, and in most specimens seen in dorsal or ventral view they appear to lie one directly behind the other.

In all other structural details dealt with by Fischoeder the writer's specimens show conformity, so that further comments would be superfluous.

Gastrothylax crumenifer (Creplin, 1847)

Synonym: *G. compressus* Brandes, 1898

A few of the 180 specimens from the rumen of the buffalo were identified readily as belonging to this species, in which the testes lie side by side and the uterus runs on opposite sides of the sagittal plane in anterior and posterior halves of the body, crossing this plane somewhere near the middle of the body.

In identifying *G. compressus* with *G. crumenifer*, Maplestone (1923) paid attention to points of difference between them, but found in a collection of several hundred specimens many which could be identified as belonging to either species. He concluded that previous separation had been effected on points of individual variation. Fukui obtained *G. crumenifer* only once (from

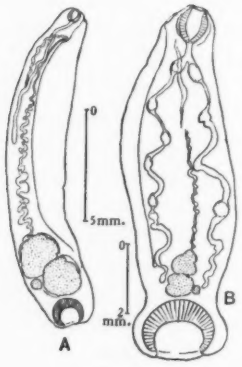

Fig. 1.

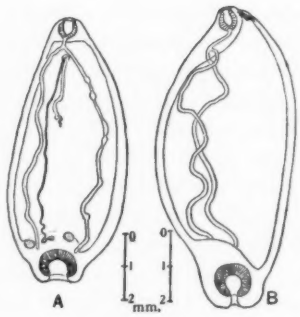

Fig. 2.

Fig. 1. A, *Gastrothylax elongatus* from rumen of the ox (side view); B, *Gastrothylax cobboldii* from oesophageal groove of the cow (dorsal view).

Fig. 2. *Gastrothylax spatiosus*. A, from rumen of the buffalo (dorsal view); B, from rumen and reticulum of "Siam bull" (side view).

Formosa) but accepts Maplestone's synonymy, as does Travassos. The writer's specimens fit the description of *G. compressus* rather than that of *G. crumenifer* as accepted by Fischoeder. They are 7–8 mm. long, that is to say, much smaller than either species as described by Fischoeder, and 4·7 mm. in maximum width. The posterior sucker is 1·8 mm. in diameter and its dome 1·7 mm. high. The caeca are fairly straight and the uterus is less convoluted than in Fischoeder's *G. crumenifer*. In the matter of the size of the eggs, the specimens more nearly resemble *G. compressus*; mean measurements of six eggs (which varied only slightly in size) are 0·116 × 0·060 mm. Fischoeder's figures for *G. compressus* and *G. crumenifer* being 0·115–0·125 × 0·060–0·065 mm. and 0·125–0·135 × 0·065–0·070 mm. respectively. The testes are much larger than those shown in Fischoeder's Fig. 48, and in size and lobing more closely resemble those

shown in his Fig. 44 (*G. crumenifer*). Maplestone's statement appears to settle the question of synonym, however, and the writer accepts his ruling in considering the specimens as *G. crumenifer*.

Gastrothylax spatiosus Brandes, 1898

Synonym: *G. cruciformis* Leiper, 1910

Fischoeder (1902–3) separated the species of the genus *Gastrothylax* on the shape of the ventral pouch as seen in transverse section, arranging them in the 5 groups shown below:

Transverse section of pouch:

(1) Triangular, with undivided apex dorsal (*crumenifer* and *compressus*).

(2) Triangular, with bifurcated apex dorsal (*gregarius*).

(3) Circular (*spatiosus*).

(4) Triangular, with undivided basal angles dorsal and apex ventral (*synethes*, *elongatus*, *cobboldii* and *mancupatus*).

(5) Triangular, with bifurcated basal angles dorsal and apex ventral (*minutus*).

Maplestone investigated the form of the pouch in several species of the genus *Gastrothylax*, including 20 specimens of a species which he regarded as *G. spatiosus*, finding it to vary greatly in different individuals and even in different regions of the same individual. Consequently, he deemed it of no value in specific diagnosis, and regarded Fischoeder's species *synethes*, *mancupatus* and *minutus* as synonyms of *G. spatiosus*. At the same time he accepts Leiper's *G. cruciformis* as a genuine species and separates it from other species by the five angles shown by the ventral pouch in transverse section.

The following material was examined by the writer:

22 specimens from the rumen and reticulum of the "Siam bull" (16).

2 specimens from the liver of the "Siam bull" (30).

Many of the 180 specimens from the rumen of the buffalo (17, 20, 21).

The specimens are all remarkable for the capaciousness of the ventral pouch and the thinness of its walls, and for the great length and slenderness of the caeca (Fig. 2). They are 4–11 mm. long and slightly compressed laterally so that an individual 10·5 mm. long measures 5·2 mm. dorso-ventrally but only 3·6 mm. from side to side. The ventral outline of the body is much straighter than the dorsal, which in some specimens is strongly convex (Fig. 2 B).

The parts of a number of specimens were carefully measured and the following representative data were obtained. The body of a specimen 8·4 mm. long measures 4·1 mm. dorso-ventrally. The pouch measures 3·4 mm. dorso-ventrally, and its dorsal and ventral walls are of unequal thickness (0·4 and 0·3 mm. respectively). The oral sucker is almost spherical and 0·7 mm. in diameter, the posterior sucker faces posteriorly and is 1·1 mm. in diameter and domed to about 1·0 mm. The oesophagus runs postero-dorsally and is very little longer than the oral sucker. From its posterior end extremely narrow

caeca (not more than 0·1 mm. wide) run back in a series of waves which sweep dorso-ventrally as well as from side to side and reach as far back as the level of the posterior sucker. The caeca are located in the dorso-lateral part of the body.

In many specimens examined the testes are difficult to make out because immature, but in whole mounts which have been flattened somewhat they can be seen as small ovoid bodies lying on either side of the middle line and separated from one another by roughly the diameter of the posterior sucker (Fig. 2 A). The ovary and ootype lie almost if not quite in the middle line of the body, and the uterus is extremely slender and runs an almost straight course anteriorly. Only one point about the male reproductive organs calls for mention; the pars musculosa is almost straight. The genital pore is situated 0·2 mm. from the anterior end of the worm.

The brief description given above shows that the writer's specimens agree closely with Fischoeder's *G. spatiosus*, the chief differences in his specimens being the slightly shorter and straighter caeca and their slightly more lateral position, and the convoluted pars musculosa. These differences may be attributed to individual variations. Comparison of Fig. 2 with Fischoeder's Fig. 51 will convince the reader that one and the same species is being considered in the two instances. Further, comparison of Maplestone's Fig. 17 and 18 with either of the above figures will show that Maplestone's specimens do not belong to this species. It is difficult to decide as to which species he examined, but from his figures it may be judged that in all probability he was dealing with rather large specimens of Fischoeder's *G. minutus*. In the writer's opinion it would be difficult to find two more dissimilar members of the genus *Gastrothylax* than Fischoeder's *G. spatiosus* and either *G. minutus* or Maplestone's specimens.

For the reasons that the ventral pouch is so capacious and round or oval in transverse section and the caeca are so narrow, the writer would separate *G. spatiosus* as a distinct species from the forms which Maplestone regarded as synonymous with it. On the other hand, the form which Leiper described as *G. cruciformis* must be considered identical with *G. spatiosus* as here recognised. Leiper's specimens measured (allowing for the misprint in his text) 5–8 mm. in length and were immature. The peculiar shape of the ventral pouch as seen in transverse section Leiper explains in the following words: "In transverse section the cavity maintains a distinctly pentagonal shape in all degrees of contraction of the body; the base of the pentagon lying parallel to and upon the ventral surface, and the apex projecting towards the dorsal side of the parasite. In certain conditions of contraction the two sides that form the apical angle and the side that forms the base frequently buckle so as to produce the figure of a cross; under no circumstances are the five angles obliterated." The "buckling" is not represented in the diagram illustrating this cross-like sectional shape of the pouch; instead, the body is shown as being oval in outline. It may be concluded that the pentagonal shape is the less distorted and more natural one, while it also very closely resembles the circular outline seen in

G. spatiosus. In other essential characters, Leiper's specimens bear a close resemblance to *G. spatiosus*; the extremely fine caeca, for instance, "have a width of 0·06 mm. and follow a wavelike course to end at the level of and ventral to the testes".

Gastrothylax synethes Fischoeder, 1901

Synonyms: *G. mancupatus* Fischoeder, 1901
G. minutus Fischoeder, 1901
G. bubalis Innes, 1912
Wellmanius wellmani Stiles & Goldberger, 1910

The species of Fischoeder, Innes, and Stiles & Goldberger which Maplestone identified with *G. spatiosus* may, for the present, be considered synonyms of *G. synethes*, subject to further investigation.

NOTES ON THE GENUS *PARAMPHISTOMUM* FISCHOEDER

At one time or another since the commencement of the current century no less than 33 species have been claimed for the genus *Paramphistomum*. Fischoeder (1901, 1903, 1904) recognised 12 species, Leiper (1910) described 6 new species, Stiles & Goldberger (1910) added 8 more to the list, Faust (1920) still another, Railliet (1924) 2 more, Fukui (1926, 1929) 3 more, one of which he relegated to synonymy, and Holl (1930) completes the list with still another. This list does not include forms like *P. papillostomum* MacCallum, 1916 [now considered synonymous with *Schizamphistomum scleroporum* (Creplin, 1844)] and *P. aspidonectes* MacCallum, 1917 (now *Opisthoporus*).

Stiles & Goldberger separated from Fischoeder's genus *Paramphistomum* the new genus *Cotylophoron*, containing Fischoeder's species *cotylophorum* and the new species *indicum*, because of the presence of a sucker bordering the genital pore. Maplestone accepted the genus but regarded the latter species as a synonym of the former. With regard to Leiper's two species, *minutum* and *sellsi*, which are referred to this genus, Maplestone identified the latter with the former, but Stunkard (1925) and Fukui (1929) deem them both good species. The writer accepts *sellsi* as a good species but identifies *minutum* with *cotylophorum*.

Fischoeder (1903) linked with *P. cotylophorum* three others, to him, distinct species of the genus on account of the lateral position behind the excretory pore of the opening of Laurer's canal. Of these species, Maplestone identified two (*microbothrium* and *bothriophoron*) with *P. cervi* and the remaining one (*calicophorum*) with *P. explanatum*. Fukui (1929) deems the genital atrium of *bothriophoron* of specific importance. The writer regards *bothriophoron* as a synonym not of *P. cervi* but of *P. cotylophorum*, in view of the great variability in the development of the genital sucker, genital atrium and vitellaria. Stunkard (1929) observed this variability in specimens from the Belgian Congo without attaching importance to it.

Despite variability in the relative positions of the excretory pore and the opening of Laurer's canal observed by Stunkard (1929), there seems to the writer to be no more reason for attributing generic rank to *P. cotylophorum* on account of its genital sucker, than for giving generic status to *P. orthocoelium* on account of the nature of the gut or vitellaria. The writer would suggest that the genus *Cotylophoron* be relegated to synonymy with the genus *Paramphistomum*.

Further points concerning the species of *Paramphistomum* may be mentioned here though discussed in detail later. To the species regarded by Maplestone as synonyms of *P. cervi* the writer would add *P. liorchis*, previously regarded as a good species, on account of the smooth boundary of its testes, *P. pisum*, which Fukui has given good reasons for believing identical with *P. cervi*, *P. ichikawai*, which is separated from *P. cervi* because of apparently unimportant differences in the genital papilla, and *P. birmiense*. Railliet's second species, *microon*, so named (1924) on account of the small size of its eggs as measured by Evans & Rennie (1908), shows the characters of *P. cervi* but calls for further investigation. To the species regarded by Maplestone as synonyms of *P. explanatum* the writer would add *P. gigantocotyle*, *P. formosanum* and *P. ijimai*, for reasons to be given presently. Difficulties in distinguishing between *P. cervi* and *P. explanatum* render doubtful even the distinctness of these species. Species of the genus *Paramphistomum* may be identified by the following key.

KEY

1. Body leaf-like; testes side by side ... ... **buxifrons**
   Body not leaf-like; testes directly or obliquely one behind the other **2**

2. Genital sucker present **3**
   Genital sucker not present **4**

3. Oral and posterior suckers both fairly large ... ... **cotylophorum**
   Oral and posterior suckers small ... ... **sellsi**

4. Oral sucker almost spherical **5**
   Oral sucker hour-glass shaped; papillae and spines in anterior division ... **gotoi**

5. Oesophagus long; caeca wide; excretory canal does not cross Laurer's canal **orthocoelium**
   Oesophagus short; caeca narrow; excretory canal crosses Laurer's canal **6**

6. Testes one directly behind the other **7**
   Testes obliquely situated; posterior sucker large ... ... **explanatum**

7. Testes rounded; posterior sucker small ... ... **cervi**
   Testes transversely elongated; posterior sucker relatively very large ... **siamense**

Paramphistomum cervi (Schrank, 1790) and **Paramphistomum explanatum** (Creplin, 1849)

Synonyms

P. gracile Fischoeder, 1901
P. microbothrium Fischoeder, 1901
P. bathycotyle Fischoeder, 1901
P. epiclitum Fischoeder, 1904
P. papillosum Stiles & Goldberger, 1910
P. papilligerum Stiles & Goldberger, 1910
P. indicum Stiles & Goldberger, 1910
P. liorchis Fischoeder, 1901
P. pisum Leiper, 1910
P. ichikawai Fukui, 1922
P. birmiense Railliet, 1924
?*P. microon* Railliet, 1924
P. calicophorum Fischoeder, 1901
P. crassum Stiles & Goldberger, 1910
P. cauliorchis Stiles & Goldberger, 1910
P. fraternum Stiles & Goldberger, 1910
P. gigantocotyle (Brandes, 1896)
P. formosanum Fukui, 1929
P. ijimai Fukui, 1922

These two species are linked together for reasons which will be given presently. First of all, it is necessary to comment briefly on some general characters and on the synonymy.

As accepted by Fischoeder, *P. cervi* is 5–11 mm. long. The posterior sucker is 1/5–1/4 of the length of the body in diameter, the coarsely-lobed testes lie one behind the other, the vitellaria are well developed and composed of irregularly arranged follicles, the genital pore lies near or behind the bifurcation of the gut, and the excretory pore lies in front of the opening of Laurer's canal, which crosses the excretory canal.

According to Maplestone, the synonyms of this species include four species of Fischoeder's and three of Stiles & Goldberger's (see the synonymy given above). Fukui suggests that *P. pisum* must be added to the synonymy. Leiper acknowledges that his specimens showed "a certain amount of contraction", which explains what at first sight appear to be characters of specific importance, for instance, the deep retraction of both suckers, the relative shortness and wideness of the oesophagus (and also its transverse folds), the wideness of the caeca, the relatively large size of the testes as seen in sagittal section, and the spiral coiling of the pars musculosa. The unusual habitat (small intestine) may be explained on the assumption that the specimens were collected prior to migration into the stomach, as happens with *P. cotylophorum* (le Roux, 1930). The length of the specimens is not stated but, judging by the sizes of measured parts in Leiper's diagram, we can conclude that they were small (6–7 mm.).

P. liorchis is included in the synonymy for the following reasons. According to Fischoeder, *P. liorchis* differs from *P. cervi* in its smaller size (3–8 mm.) and smoother testes. Maplestone considered the smooth outline of the testes of specific importance but asserted that "in young worms (*P. cervi*), and even in fully extended examples with eggs in the uterus, there is sometimes a distinct interval between the two testes up to as much as 1 mm. In these instances the testes are circular in outline." He adds, "the testes are always divided into lobes, which are more distinct in young worms than in older ones".

The writer submits that when Fischoeder separated *P. cervi* and *P. liorchis*, both of which had been known previously as *Amphistoma conicum*, he was considering the general outline of the testes and not the lobing, which may or may not have been masked by it. His actual words are, "die rundlichen bezw. schwach ovalen Hoden nicht gelappt sind, sondern eine fast glatte Oberfläche besitzen, an welcher höchstens nur ganz flache Unebenheiten sich bemerkbar machen". Further, the species was based on only six specimens, which do not constitute a sample sufficiently large to allow for individual variation.

Fukui admits that in such characters as the many-lobed testes, the small genital atrium, the size of the posterior sucker and the relative position of the excretory pore, *Paramphistomum ichikawai* is indistinguishable from *P. cervi*. The species are separated on account of slight differences in the genital papillae and associated ducts and in the vitellaria. In *P. ichikawai* the genital papilla is large and stout; in *P. cervi* it is rather small. Maplestone pointed out that the structure of the genital papilla and atrium was used by Fischoeder and by Stiles & Goldberger in specific diagnosis. In his study of *P. cervi* he showed that the papilla is composed almost entirely of muscle and that the atrium is enclosed by a special thickening of the subcuticular layer. Both structures, it was stressed, show greatly variable form, the papilla being capable of retraction into the body, with consequent deepening of the atrium. The form of the atrium and the size and shape of the papilla Maplestone deems purely matters of chance, and useless in specific diagnosis. Had Maplestone been aware of Fukui's species, which was made in 1922, he would probably not have considered it a good species. For these reasons, the writer regards *P. ichikawai* as a synonym of *P. cervi*.

The specimens described by Evans & Rennie (1908), which Railliet named *P. birmiense*, were 6–9·5 mm. long. The posterior sucker was stated to be large (2 mm. in diameter) and the testes very large and situated one in front of the other. The specimens were found in the bile duct and cystic duct, a fairly common habitat of *P. explanatum*, but the characters given above and the relatively large size of the eggs justify the writer in considering *P. birmiense* a synonym of *P. cervi*. The specimens (about 12) which Railliet named *P. microon* were 7·5–11·5 mm. long. The posterior sucker was stated to be small (1–1·5 mm. in diameter) and in characters other than the size of the eggs the specimens bore a remarkable resemblance to *P. cervi*. The eggs were stated to measure 75–87·5 × 43·75–50 μ and provide a puzzling character, because in not one of 12 species of the genus *Paramphistomum* recognised by Fischoeder were the eggs found to be smaller than 105 × 60 μ. For finality further investigation is required.

In considering *P. explanatum* mention must be made first of all of the exclusion from the synonymy of one of the species of Stiles & Goldberger which Maplestone identified with this species. This is *P. siamense*, which is regarded as a distinct species with *P. anisocotylea* as a synonym. The synonymy is discussed under the specific heading.

Fukui (1929) acknowledges close relationship between *P. explanatum* and *P. formosanum* but separates these species on account of certain differences in the microscopic structure of the oral sucker. The muscle fibres of this sucker are said to be arranged according to the same plan in members of the genera *Paramphistomum* and *Gastrothylax*, but in *Paramphistomum formosanum* "the arrangement of the muscle fibres is very loose and not so regular as in other species". The species was made for 8 specimens, 5 of which are photographed and labelled "not well fixed". The writer regards the unusual arrangement of muscle fibres as individual variations due to local contraction, and with due regard for similar characters would identify *P. formosanum* with *P. explanatum*.

P. gigantocotyle has much in common with *P. cervi* on the one hand and with *P. explanatum* on the other. It differs from *P. cervi* in having a relatively larger posterior sucker, and from *P. explanatum* in having bilobed testes lying one behind the other. Leiper's supplementary description of *P. gigantocotyle* (1910) is based on examination of specimens 8 mm. long. The posterior sucker is 4·0 mm. in external, and 3·2 mm. in internal, diameter. Leiper states: "I attribute my specimens to this species mainly on the ground of the presence of an exceedingly large ventral sucker." The testes were elongated at right angles to the main axis of the body, measured 1·1 × 0·5 mm., lay one behind the other, and were "deeply incised transversely". Leiper's Fig. 30 shows the specimens to have been greatly contracted, and to this was probably due the form of the testes. The writer relegates the species to synonymy with *P. explanatum* on the ground that he has examined specimens with a posterior sucker half the length of the body in diameter, but in which the testes are bilobed in some instances (though not in the same plane in the two gonads) and trilobed in others. Further, the testes of these specimens are not sufficiently out of line to be typical of *P. explanatum* as generally accepted, and in having to choose between the terms "oblique" or "in tandem" one would be obliged to use the latter term.

The following material has been examined:

P. cervi: 25 specimens from the large intestine (?) of the ox (15).

P. explanatum: 80 specimens from the bile duct of the buffalo (11).
70 specimens from the liver of the goat (4).
50 specimens from the bile duct of the goat (7).
6 specimens from the ox (location not given) (23).

As far as the writer is aware, there is no previous record of the goat serving as final host of *P. explanatum*.

The specimens determined as *P. cervi* are typical of this species but have a relatively large posterior sucker. They are rather more than 8 mm. long with an oral sucker that is almost spherical and 1·1 mm. in diameter. The posterior sucker measures 2·5 mm. in external diameter (about 2/7 of the length of the body) and its opening is about 1·5 mm. wide. The testes are only slightly lobed and lie one directly behind the other. The genital pore is situated exactly at

the bifurcation of the gut. Apart from these perfectly general characters, the well-known structure of the specimens does not call for any comments.

The specimens regarded as *P. explanatum* are of special interest because difficult to determine. According to Maplestone, the form of the body, which is largely determined by the size of the posterior sucker, is an unreliable criterion of distinction between *P. cervi* and *P. explanatum*. The species are generally separated by the different relative positions of the testes. In fully grown specimens of *P. cervi* "the testes lie one directly behind the other", whereas in *P. explanatum* they "are always diagonally situated one overlapping the other, both laterally and antero-posteriorly". The distinction is Maplestone's. In young specimens of *P. cervi*, however, the testes are sometimes diagonally placed, a character which is said to undergo modification with the attainment of sexual maturity.

Maplestone's generalization on variable form was the outcome of careful measurements of individuals of the two species. In 89 well-extended specimens of *P. cervi*, all belonging to the same set, the ratio of the diameter of the posterior sucker to the length of the body was found to vary between the wide limits of 1/8 and 1/3·5, all intermediate ratios being obtained. The variations were "so gradual that it was found impossible to draw a dividing line at any point in the series". Individuals from different sets showed even greater variability, extending the range of ratios from 1/2·5 to 1/9·5, truly remarkable figures.

Measurements of 10 specimens of *P. explanatum* gave similar general results. On the whole, the posterior sucker of this species is relatively larger than that of *P. cervi*, but the ratio of its diameter to the length of the body was found to vary from 1/1·9 to 1/3·6, that is to say, above and below Fischoeder's figure, which Maplestone estimates at 1/2·3. In the matter of the form of the body, therefore, *P. cervi* and *P. explanatum* have been observed to merge one into the other.

The testes of the writer's specimens do not show the diagonal positions characteristic of *P. explanatum*. They are at most only very slightly out of line (Figs. 3 and 4). Determination as *P. gigantocotyle* is unsatisfactory, because in some instances the testes are bilobed (with constrictions in the frontal and sagittal but not in the transverse plane) (Fig. 3), while in others they are trilobed. Measurements given in Table II (for the "mean" of three typical and well-extended specimens) show that the writer is obliged to determine the specimens as *P. explanatum*.

Table II

	Absolute measurements mm.				Ratio organ size/length of body			
Set no. ...	11	4	7	23	11	4	7	23
Length of body	8·2	10·2	10·0	9·2				
Maximum width	3·7	5·3	4·9	5·6	1/2·2	1/1·9	1/2·0	1/1·6
External diameter posterior sucker	3·1	3·7	3·6	4·4	1/2·6	1/2·7	1/2·8	1/2·1

There is no doubt that separation of *P. cervi*, *P. gigantocotyle* and *P. explanatum* is difficult and uncertain on account of variability of form, which may be attributed to one or more of three possible causes, (1) to valid species being relegated to synonymy, (2) to perpetuation in fixation of spasmodic contractions or relaxations of the body and to distortion due to bad fixation, and (3) to peculiarities of the growth processes.

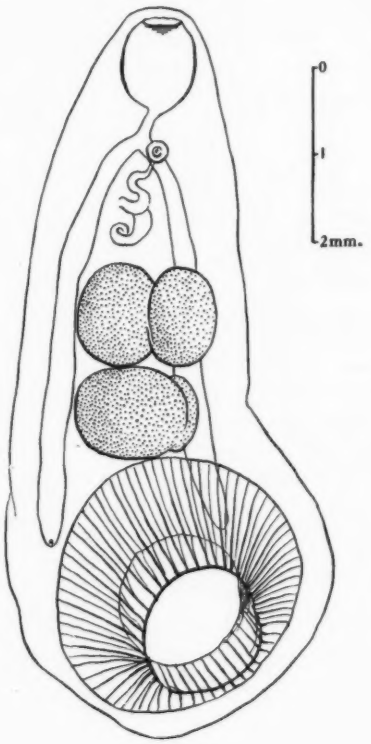

Fig. 3.

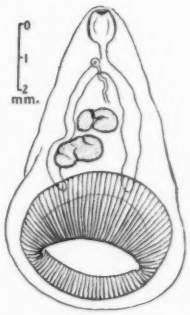

Fig. 4.

Fig. 3. *Paramphistomum explanatum* from bile duct of buffalo (set 11) (ventral view).
Fig. 4. *Paramphistomum explanatum* from ox (set 23) (ventral view).

The first and second possibilities are not nearly as important as it would seem at first sight, because the synonymy is tested by a succession of systematists, who, by the use of new specimens, become progressively more aware of misconceptions due to the effects of fixation. A few words seem desirable on the third possibility.

Many members of the Paramphistomidae show evidence of growth definitely graded along the main axis of the body, particularly the forms under considera-

tion. The large size of the posterior sucker can only be due to growth more rapid and intensive than that of the rest of the body *at some period* of post-larval life. This sucker is a *hypergonic* (positively heterogonic) organ (Dawes & Huxley, 1934), whose size is probably a function of the absolute size of the body, as has been found in other organisms showing this type of growth. It follows, therefore, that a systematist describing Paramphistomidae is omitting an important detail if he neglects to record the absolute size of his specimens. Further, we have few data which enable us to measure the effects of different environments, as represented by different hosts or different situations in the same host, on this graded growth. Assuming that the environment is capable under certain conditions of retarding growth of the body as a whole, it is unlikely that the growing parts will be equally affected. It is more likely that parts having a relatively rapid rate of growth (*e.g.* the posterior sucker) will suffer greatest retardation, with marked alteration of the proportions of the body. Where maturity is attained at small total size (as in some instances of crowding within the body of the host) the more rapidly growing parts will not have had the opportunity of reaching the relative as well as the absolute size seen in large specimens.

The form of the body must play a part in determining the positions of the testes, which may be nearly as wide as the body at maturity. If the posterior sucker is relatively large, there will be space available for the oblique arrangement of the testes. If, on the other hand, the posterior sucker is relatively small, the testes can only be accommodated one behind the other. Variable form in *P. cervi*, *P. gigantocotyle* and *P. explanatum* may determine the variable positions of the testes, though the positions of these gonads will depend in part on the sizes they attain. The writer would venture the prediction, however, that it will be necessary sooner or later to group all three species, as accepted up till the present time, under the specific title of *P. cervi*. It might be expected that the study of development would provide a solution to this problem, but so far it has proved misleading. *Cercaria pigmentata* is the recognised larval form of *Paramphistomum cervi* and, according to Grobbelaar (1922), *Cercaria frondosa* is the larval form of *Paramphistomum calicophorum*, a synonym of *P. explanatum*. Le Roux (1930) claims, however, that Faust's description of this cercaria (1919) adequately describes the larval form of *P. cotylophorum*. It is necessary therefore to await further investigation of this point.

Paramphistomum siamense Stiles & Goldberger, 1910

Synonyms: *P. anisocotylea* Faust, 1920
P. explanatum Maplestone, 1923 (in part)

The specimens which Faust named *P. anisocotylea* were ovoid (6–6·3 mm. long and 3·5 mm. wide) and the conspicuous posterior sucker took up more than half the length of the body (3·1–3·4 mm. in diameter). The oral sucker was small (0·85 mm. in diameter), the oesophagus short and the caeca inflated and

sausage-like and extending back to the level of the middle of the posterior sucker. The testes, described as subovoid or rhomboid, were said to lie one behind the other. The position of the excretory pore in relation to the opening of Laurer's canal was not noted, but Faust is no doubt correct in his belief that his specimens belonged to a species distinct from though closely allied to *P. explanatum*.

The specimens which Stiles & Goldberger named *P. siamense* measured 6–9 mm. in length and the posterior sucker in three "press preparations" 3·5, 4·0 and 5·0 mm. in diameter. The species was differentiated from *P. fraternum*, later regarded as a synonym of *P. explanatum*, on account of the form and the relatively large size of the posterior sucker.

Maplestone apparently overlooked Faust's paper, but if he had seen it he would have been compelled to admit that *P. siamense* compares much more closely with *P. anisocotylea* than with *P. explanatum*.

The writer examined 2 sets of specimens of this species taken from the small intestine of goats (sets 25 and 27), in all, 27 specimens. They are smaller than the specimens of either Faust or Stiles & Goldberger and though the testes are well developed, the absence of eggs in the uterus shows them to be immature, which may explain their occurrence in the small intestine. They are brown, somewhat shrivelled and difficult to study, but the diagnostic characters can be made out easily. The proportions of the body are scarcely distinguishable from those of Faust's specimens, though the size is markedly smaller, as the measurements (in mm.) in Table III show:

Table III

	Specimens from	
	Set 25	Set 27
Length of the body	2·4	1·2
Maximum width	1·9	0·9
Diameter of oral sucker	0·7	0·3
External diameter of posterior sucker	1·2	0·7
Internal diameter of posterior sucker	0·7	0·3

It is surely remarkable that in a paramphistomid little more than 1 mm. long the posterior sucker takes up more than half of the length of the body (Fig. 5). One might incline to the belief that very intensive graded growth has brought about in *P. siamense* limitation of total absolute size, which is much smaller than in most species of the genus.

The caeca of the writer's specimens are wide and constricted at indeterminate points. In a specimen 2·5 mm. long the maximum width of the caeca is 0·4 mm., or about one-fifth of the total width. In the same specimen, the testes are 0·5 mm. in transverse diameter but little more than half this measurement in length. In specimens mounted ventral side uppermost, the posterior testis is hidden beneath the rim of the posterior sucker. The genital pore lies just behind the oral sucker. There is no sign of a genital sucker, but

the ventral integument is greatly thickened (5 times as thick as the dorsal integument), which may be due to artificial flexure of the body.

Paramphistomum gotoi Fukui, 1926

This species was discovered in *Bos taurus* and is rare in Japan. A few specimens were found in the writer's collection and were obtained from the rumen (and the oesophagus near its opening into the rumen) of the buffalo, a newly discovered host.

According to Fukui (1926, 1929), the most peculiar character of *Paramphistomum gotoi* is the oral sucker, which is large and bears projecting into the lumen of its anterior 2/5) many large papillae with flattened apices, each

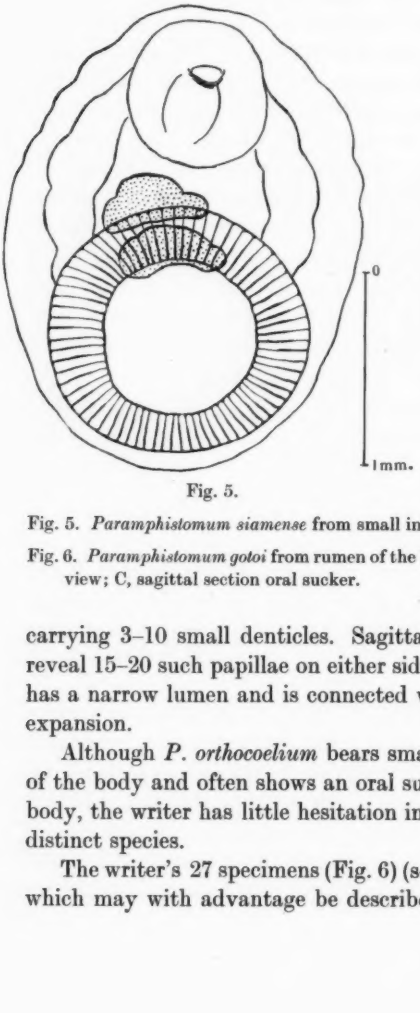

Fig. 5.

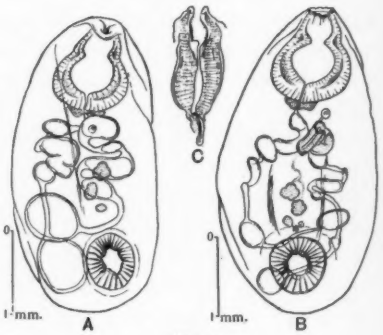

Fig. 6.

Fig. 5. *Paramphistomum siamense* from small intestine of goat (set 25) (ventral view).

Fig. 6. *Paramphistomum gotoi* from rumen of the buffalo (set 17). A and B, entire worms in ventral view; C, sagittal section oral sucker.

carrying 3–10 small denticles. Sagittal sections passing through the sucker reveal 15–20 such papillae on either side. The posterior part of the oral sucker has a narrow lumen and is connected with the anterior part by a funnel-like expansion.

Although *P. orthocoelium* bears small papillae at or near the anterior end of the body and often shows an oral sucker considerably retracted within the body, the writer has little hesitation in agreeing with Fukui that *P. gotoi* is a distinct species.

The writer's 27 specimens (Fig. 6) (sets 17 and 26) show certain peculiarities which may with advantage be described briefly. They are 3·5–5·1 mm. long

and were at first thought to constitute a new species, the oral sucker being of relatively large size and clearly divided into anterior and posterior parts. The examination of several series of sections demonstrated the absence of pouches and clearly showed the papillae within the anterior division. The writer could not make out spines. The entire sucker is pear-shaped and its length is equal to the maximum width of its posterior division, or 1/5–1/4 of the length of the body (Fukui's determination is 1/6). The posterior sucker, which faces ventrally, is distinctly smaller than the oral, its diameter being 1/6–1/5 of the total length (as against Fukui's determination of 1/5–1/4).

The anterior division of the oral sucker is only one-third of the entire length and its diameter little more than half that of the posterior division. Serial sections show that the lumen of the sucker is a very wide but shallow crevice. The numbers of papillae are not significantly different from those given by Fukui.

The oesophagus is extremely slender and a pad of muscular tissue marks its origin at the hinder border of the oral sucker. It runs for a short distance postero-dorsally. The caeca are alternately constricted and dilated at certain points. Some of the swellings in a specimen only 3·6 mm. long are at least 0·7 mm. wide, but smaller swellings were noted in larger specimens. The caeca are much folded, especially in the transverse plane, some of the folds extending inwards almost to the middle line. The most peculiar features of the caeca, however, are the dilated terminal portions, which in some specimens lie on the opposite side of the body. There is a possibility of mistaking them for other organs, because the constricted part of each caecum between the ultimate and penultimate swellings is extremely narrow and resembles a fine fibrous cord.

In the writer's immature specimens, the testes are small (no more than 0·3 mm. in diameter) and 3- or 4-lobed. They lie one in front of the other just behind the middle of the body, the anterior testis slightly to the right, the posterior slightly to the left of the middle line. The ovary and ootype are situated a little further back and just in front of the anterior margin of the hinder sucker. The vitellaria are only feebly developed. The well-developed retracted cirrus is coiled into 1½ loops and the genital pore is situated just in front of the fork of the gut.

Serial sections show the excretory canal to be very short and situated near the posterior end of the bladder. Laurer's canal curves round the anterior end of the bladder and opens far in front of the excretory pore. This departure from Fukui's description can only be explained on the assumption of variability. There can be no question about the determination, which is based on the structure of the oral sucker.

Paramphistomum cotylophorum Fischoeder, 1901

Synonyms: *Cotylophoron indicum* Stiles & Goldberger, 1910
Paramphistomum bothriophoron (Braun, 1892), Fischoeder, 1901
P. minutum Leiper, 1910

Having been recorded fairly recently by Tubangui (1933) as occurring in the Philippines and by Krull (1934) in Puerto Rico, this species is now known to occur in three continents. Growing realisation of its importance and also of that of other Paramphistomids is shown in the work of Le Roux (1930) and Krull (1934). Le Roux has shown that the parasite is decidedly pathogenic in sheep, that the cercariae and young flukes are found in the duodenum, and that the immature flukes migrate into the rumen to mature and oviposit, as Looss long ago believed might be the case. The bearing of this work on puzzling records of the location of immature flukes will be obvious; what appears to be an incongruous situation for the adult is the natural one for the young fluke. Krull has made critical measurements of the eggs of this species, which can easily be mistaken for the eggs of *P. cervi*. Valuable data bearing on the duration of the life cycle are to be found also in his paper.

The writer's material comprised:

30 specimens from the rumen of the ox (18).

80 specimens from the rumen of the goat (22).

The goat was not known to serve as host to this species until fairly recently, when Szidat (1932) established the fact for specimens collected in Africa.

The specimens show differences which may be of significance to the systematist. Those from the ox are typical specimens as described by other writers, 4·8–6·0 mm. long and with fleshy bodies suitable for examination only when sectioned or bisected. The oral sucker is 0·8–1·1 mm. long and 0·6–0·7 mm. wide, the posterior one 1·9–2·0 mm. long with a wall 0·3–0·45 mm. thick. The vitellaria are extremely well developed and obscure most other organs in bisected individuals. Even unstained specimens examined entire in alcohol show great variation in the condition of the genital sucker and atrium. In some specimens the sucker is almost completely retracted within the body, a small opening alone remaining visible. In other specimens the genital sucker protrudes considerably from the ventral surface of the body and the genital papilla projects well beyond the limits of the sucker. The anatomy of this species, as described by Fischoeder and commented upon by Stunkard (1929), is so well known that restatement of its details is unnecessary.

Some attention must be paid to the specimens from the goat. They are 5·2–5·4 mm. long, possess each an almost spherical oral sucker 0·7–0·8 mm. in diameter, and a posterior sucker 1·6 mm. in diameter and are so strongly domed as to measure 1·1–1·2 mm. from crown to opening. The genital opening is prominent and bordered by a genital sucker which is small (0·7–0·8 mm. long and 0·9 mm. wide) and has only a thin muscular layer. The vitellaria are feebly-developed and the testes are small (0·5 mm. wide and 0·35 mm. long)

and lie one behind the other but diagonally placed. A subtle difference between these specimens and those from the ox is the altogether looser texture of the body, which lends itself to the preparation of perfectly clear whole mounts.

Fischoeder separated *P. bothriophoron* from other species of the genus in which the excretory pore occupies a lateral position on account of the large "Vorraum" into which the genital pore opens. In the four specimens (6–9 mm. long) and two series of sections he examined, this space (here referred to as genital atrium) was 0·7 mm. deep and 1·04 mm. wide. Its muscular wall, judging by the figures, must have been at least 0·1 mm. thick, that is to say, at least half as thick as the genital sucker of *P. cotylophorum*. The wall of the atrium merges gradually into the wall of the body outside it.

Maplestone examined serial sections and whole mounts of many specimens of *P. cotylophorum* and found that "differences in size, degree of extrusion or retraction of the genital sucker and papilla, with consequent changes in the genital atrium, were almost as numerous as the specimens examined". The specimens "show such great differences in appearance that at first sight it might be considered that different species are being dealt with". The atrium, even when large (his Fig. 8B), is relatively smaller than that shown for *P. bothriophoron* in Fischoeder's Fig. 25, but the thickness of the musculature of the sucker is only half that in specimens with a small atrium (Maplestone's Fig. 8 A1 and A2). If we visualise a slightly larger atrium than is shown in Fig. 8B, with corresponding attenuation of the muscular wall of the sucker, we have a close approximation to the condition seen in *P. bothriophoron*. This species has many characters in common with the specimens of *P. cotylophorum* from the goat examined by the writer.

The genital sucker has been regarded as an important character in the separation of *P. cotylophorum*, but it is known to vary greatly in different individuals, increasing in size with age, just as the vitellaria increase the numbers of their follicles. In considering a limited number of individuals one is unable to form a reliable judgment of the extent of variability. For this reason, the writer tentatively submits that Fischoeder's specimens of *P. bothriophoron* (which were, incidentally, Braun's (1911)) were specimens of *P. cotylophorum* in which the large size of the genital atrium masked the importance of the relaxed sucker bordering it.

Leiper's specimens of *P. minutum* were small (2·7 mm. long), but the uterus contained eggs. In some respects, and especially in the structure of the genital atrium, the specimens were said to differ from other Paramphistomids. The genital atrium had a muscular wall 0·1 mm. thick and sharply marked off from the surrounding parenchyma. The testes were small but situated one in front of the other in the middle line. The vitellaria formed two rows around the caeca. Laurer's canal did not cross the excretory canal and opened in front of the excretory pore. This last character provides the most serious objection that can be offered to the proposed synonymy but, as Stunkard (1929) has shown,

Laurer's canal may open in front of, at the side of or behind the excretory pore. The relative positions of the pores are not constant enough to be important.

Paramphistomum orthocoelium Fischoeder, 1901

Synonyms: *P. streptocoelium* Fischoeder, 1901
P. dicranocoelium Fischoeder, 1901
P. scoliocoelium Fischoeder, 1904
P. shipleyi Stiles & Goldberger, 1910
P. parvipapillatum Stiles & Goldberger, 1910
P. wagandi Leiper, 1910

Subsequent researches have substantiated Fischoeder's statement that "die hauptsächlichsten Charaktere dieser Art liegen im Verdauungsapparat und in der Anordnung der Dotterstöcke". In specimens 5–11 mm. long, but mostly 7–8 mm. long, which Fischoeder examined, the elongated oral sucker was 1·0–1·2 mm. long and 0·5–0·8 mm. wide. The oesophagus was at least twice as long as the oral sucker and the caeca were very wide, with lumina 0·5–0·6 mm. in transverse and 1·0–1·2 mm. in dorso-ventral measurement. The posterior sucker was small and subterminal. Further differences from *P. cervi* were the coarser vitellaria (built up of fewer and larger follicles), the constant cuticular papillae around the mouth, and the position in front of the excretory pore of the opening of Laurer's canal.

Maplestone separates this species from others on account of the testes having more than two lobes, and, with due regard for the relative positions of the excretory pore and the opening of Laurer's canal, would include five other former species as synonyms. Fukui and Travassos have accepted the synonymy and the writer agrees with them, but believes that *P. wagandi* Leiper, 1910 must be added to the list.

Maplestone's opinion of the validity of *P. wagandi* is based on a single character, the smooth outline of the testes. Leiper did not specially claim that such is the case and, in fact, makes no mention of this character in his text, but renders it apparent in his Text-fig. 35. Leiper examined only a few specimens of his species. These measured 3·5 mm. in sectional length, possessed relatively small oral suckers (0·3 mm. long and 0·14 mm. in maximum width) and in other respects conform with *P. orthocoelium* in structure. The nature of the caeca (especially in straightness, length and width) and of the vitellaria are not mentioned in Leiper's description. Careful comparison of Leiper's Text-fig. 35 with Fischoeder's Fig. 16 (one of which, apart from conventionalities, might be the mirror image of the other) convinces the writer that *P. wagandi* is none other than *P. dicranocoelium*, accepted as a synonym of *P. orthocoelium*.

The writer's specimens comprised:

100 mature specimens from the rumen and reticulum of a "Siam" bull (19).
30 immature specimens from the small intestine of a calf (24).
2 mature specimens from the large intestine (?) of a goat (19).

The last-named host has not been recorded hitherto.

The specimens from the bull were readily identified from the description and figures of Fischoeder, the relatively very wide caeca and the coarse vitellaria being the most conspicuous features of the anatomy (Fig. 7 A). Specimens 10 mm. long and 4 mm. in maximum width have caeca almost 1·0 mm. wide, which reach almost to the anterior limit of the posterior sucker. The testes are ovoid and of much smoother outline than Fischoeder indicated for his own specimens. In some well-extended specimens, the oesophagus is 2·0 mm. long and twice the length of the oral sucker, which is deeply retracted in some specimens. In less-extended specimens, with the oesophagus greatly shortened by muscular contraction, the length of this region of the gut is about equal to the length of the sucker, but the width is greatly increased and is at least twice that seen in well-extended specimens.

The specimens from the calf are not more than 6 mm. long and are distinctly juvenile. Their determination is not established beyond question, because the vitellaria are very feebly developed, but is reasonably certain on account of the long oesophagus and the relatively very wide caeca, which are 0·6 mm. or quite one-third of the maximum width of the body in diameter (Fig. 7 B).

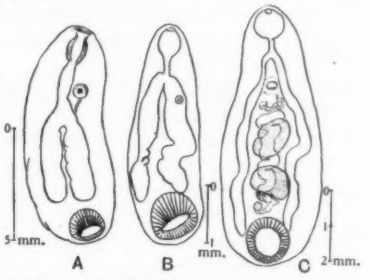

Fig. 7. *Paramphistomum orthocoelium*. A, from rumen and reticulum of the "Siam bull"; B, from small intestine of the calf; C, from large intestine(?) of the goat (sets 19, 24 and 28) (all in ventral view).

The writer experienced some little difficulty in identifying the specimens from the goat. They are little more than 7 mm. long, have almost straight or only slightly sinuous caeca measuring 0·5 mm. in maximum width and reaching to the posterior sucker, ovoid and coarsely lobed testes and a genital pore lying some distance behind the fork of the gut. One specimen has cuticular papillae at the anterior end of the body, as found in the writer's other specimens of *P. orthocoelium*, but the other lacks them. Of the forms regarded as synonyms of *P. orthocoelium* by Maplestone, the specimens most closely resemble Fischoeder's *P. streptocoelium*. In the size of the eggs, however, (0·134–0·135 × 0·73–0·80 mm.), the specimens more nearly resemble *P. scoliocoelium*. In view of the limited amount of material available for study, it is suggested that the differences observed do not warrant the separation of these specimens from *P. orthocoelium*.

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A REVISION OF THE SUBFAMILIES OF THE STREBLIDAE AND THE GENERA OF THE SUBFAMILY STREBLINAE (DIPTERA ACALYPTERAE) INCLUDING A REDESCRIPTION OF *METELASMUS PSEUDOPTERUS* COQUILLETT AND A DESCRIPTION OF TWO NEW SPECIES FROM AFRICA

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From the Wellcome Entomological Field Laboratories, Wellcome Bureau of Scientific Research, London

(With 5 Figures in the Text.)

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Introduction

In a very important revision of the Streblidae, Speiser (1900) classified them into two subfamilies: (1) the Nycteriboscinae and (2) the Streblinae. To the first subfamily he referred all the genera of the world, with the exception of *Strebla* and *Euctenodes*. Upon the presence of the ctenidium and some other characters these two American genera were included in the second subfamily. This classification of the subfamilies was also adopted by Kessel (1924, 1925).

During my study of the Streblidae I came to the conclusion that this classification is not satisfactory. It has been found that structurally the American genera of the subfamily Nycteriboscinae resemble the Streblinae far more than the Nycteriboscinae of the Old World.

In the study of the phylogeny of these parasitic Diptera the generalised characters are of more importance than those produced by convergent evolution. The ctenidium has been produced by convergent evolution, not only in this family and in the Nycteribiidae, but also in insects of other orders. It is present in Aphaniptera, in the Polyctenidae of the Hemiptera, and, in a lower degree of development, in the Platypsellidae of the Coleoptera. All these insects, with the exception of some Aphaniptera, infest mammals. They have a similar environment and are therefore constantly exposed to the same micro-

climatic factors. Of these factors, the mechanical factor appears most to have influenced the modification of the structure of the body of these ectoparasites. As the action of this factor has admittedly been the same, whatever the host, it is not surprising to find a similarity in the form of the body, or the presence of the same structure, such as the ctenidium, in insects of different orders. The presence of the ctenidium cannot therefore be used as a character in the classification of the subfamilies of the Streblidae. In a new classification of these Diptera I retain the subfamily Streblinae, because the genera of this subfamily, besides the presence of the ctenidium, differ in several other characters from the rest of the American genera. All the other American genera I separate from the Nycteriboscinae of the Old World into a new subfamily Trichobiinae. The characters upon which the classification of the subfamilies is based are discussed in detail in the following section of this paper.

In that section I do not consider the structure and the systematic position of the following doubtful Streblidae: *Brachytarsina flavipennis* Macquart (1850), *Megistopoda pilatei* Macquart (1852) and *Strebla avium* Macquart (1854). None of these Streblidae has ever been recorded since the original descriptions were written. As regards *Brachytarsina flavipennis*, I quite agree with Speiser (1908) that this species may be identical with *Nycteribosca africana* Walker, which is the commonest species in Africa. The only character upon which Macquart's species can be separated from *N. africana* is the absence of the third longitudinal vein, but this vein is very thin, pale and often indistinct in dried specimens. The original description of *Megistopoda pilatei* is quite inadequate. It is impossible to understand whether it is a streblid or a nycteribiid. Its palps are described as cylindrical, but all the known Streblidae have leaf-like palps: it is one of the characters of this family. Moreover, the position of the head, as shown in the drawing, is typically nycteribiid. It seems to me that this species may be identical with one of the species of the genus *Basilia* of the Nycteribiidae.

Strebla avium represents a rather doubtful exception; it is the only streblid which has been recorded from birds, doves and parrots. As regards this record, I suspect an error on the part of the collector. The other characters upon which the separation of this species from *Strebla vespertilionis* Fabr. is based have no specific value.

I take this opportunity to express my thanks to Dr J. C. Bequaert, of the Harvard University Medical School, for the loan of his interesting collection of the Streblidae, and also for his permission to describe two new species from Africa, which were found among the specimens. I also express my thanks to Major L. H. Dunn for the loan of two specimens of *Metelasmus pseudopterus*, and to Dr C. H. Curran of the American Museum of Natural History for the loan of the paratype of *Eldunnia breviceps*.

General morphology of the Streblidae and the characters of their subfamilies

An examination of the members of the different genera shows that the head of these Diptera has undergone much greater modification than any other region of the body. The structure of the head and the homology of its different parts have been discussed elsewhere (Jobling, 1929). During the adaptation of these Diptera to the parasitic habit of life, the head followed the modification of the body form. This modification in its turn affected greatly the position and the structure of the different parts of the head.

In the most generalised genus *Nycteribosca* the structure of the head resembles more or less that of the head of the Diptera Acalypterae (Fig. 1 A and D). It is convex dorsally and its vertex is subdivided into subregions which do not differ structurally very much from those of the Acalypterae. In the other genus of the Old World, *Raymondia*, the head is dorso-ventrally flattened, but its dorsal surface and especially the vertex has the same structure as that of the genus *Nycteribosca* (Fig. 1 B).

An examination of the head of the most generalised American genus *Trichobius* shows that it differs structurally from that of the Old World genera (Fig. 1 E). Its laterovertices (*lv.*) including the eyes (*e.*) are separated by a membrane (*m.*) posteriorly and laterally from the other regions, and only the dorsal part of the occipital region (*oc.*) is convex dorsally. This structure of the dorsal surface of the head is present in all the American genera, and is much more distinct in the species of the genera *Paradyschiria*, *Aspidoptera* and *Pterellipsis*. This difference in the structure of the head is one of the important characters upon which the separation of the American genera from the Nycteriboscinae of the Old World is based. The head of the American genera *Eldunnia*, *Strebla*, *Euctenodes* and *Metelasmus* belong to the different type; it is depressed, so that its dorsal and ventral surfaces are flat, and its posterior part is as broad as the anterior margin of the thorax. The laterovertices (Fig. 3 A, *lv.*) of these genera are also separated by a membrane from the other regions of the head, but there is another membranous space which separates the dorsal part of each postgenae (*pg.*) from the occiput (*oc.*). This membrane extends also across the ventral surface of the head, where it separates the ventral portion of the postgenae into two parts, and bears the spines of the ctenidium (Fig. 3 B). The head of these genera has become so greatly modified that it has completely lost its dipterous appearance. In the three genera of this subfamily, *Strebla*, *Euctenodes* and *Metelasmus*, the palps have followed the modification of the head and have become broad basally, concave, and triangular in outline. Their outer edges are bent dorsally and are festooned. The palps of these genera form together a triangular shield in front of the head, which reduces the resistance of the hairs during the movement of the parasites on their hosts, and in this respect may be compared with the bow of a boat.

Neither the proboscis nor the eyes are of importance as regards the classi-

fication of the subfamilies. The structure of the external part of the proboscis, *i.e.* the labium, is apparently adapted to the different structure of the skin of the bats, since the length of the labella varies very much, not only in the different genera, but also in different species of the same genus.

The eyes (*e.*) also vary greatly in their structure. They are absent in the genus *Raymondia*, are single-faceted in the genus *Nycteribosca*, while in many

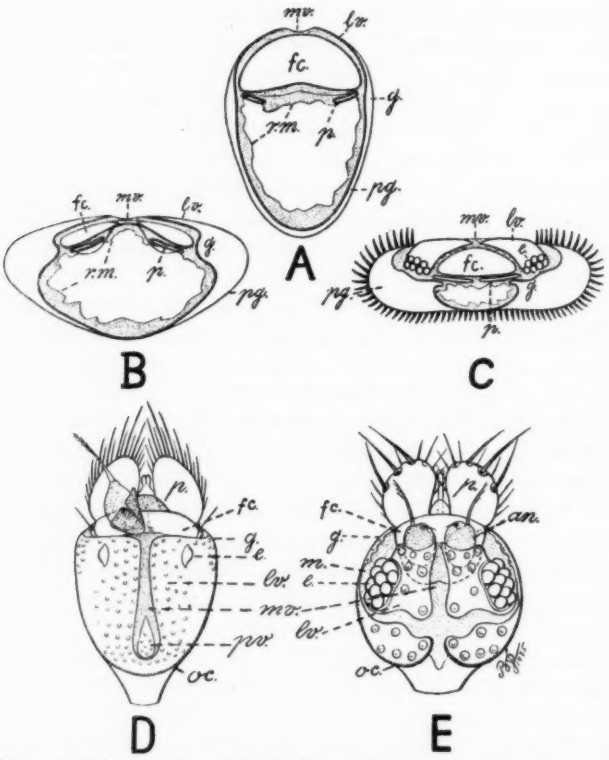

Fig. 1. A, diagram of frontal view of head of *Nycteribosca amboinensis*; B and C, same of *Raymondia lobulata* and *Strebla vespertilionis* respectively, in these figures proboscis not shown. D, dorsal view of head of *Nycteribosca amboinensis*; E, same of *Trichobius dugessii*. In all figures membranous parts stippled.

American genera they are formed by a number of facets. It is impossible to define the degree of specialisation in these Diptera by a comparative study of the structure of the eyes. For instance, the species of the genus *Nycteribosca* have single-faceted eyes, but that does not mean that the species of this genus are more specialised than those of the genus *Strebla*, or *Euctenodes*, in which the eyes are composed of a number of facets. The species of the former genus resemble Diptera Acalypterae, whereas the species of the two latter genera

rather have some resemblance to the Polyctenidae. The degree of modification of the structure of the eyes, or their complete disappearance, depends upon the exposure of the species to light, the intensity of which may vary greatly according to the habit of the different species of bats.

Another important character upon which the separation of the American species from the Nycteriboscinae of the Old World is based is the structure of the wings. The wings of the Old World genera *Nycteribosca* and *Raymondia* have five longitudinal veins and two cross-veins (Figs. 4, 5). The longitudinal veins are not uniformly setous and are not parallel, so that the cells formed by them have an irregular shape. The membrane of the cells bears setulae which are very numerous on the wings of the genus *Nycteribosca*. The alula (*al.*) is usually present and is very well developed in *Nycteribosca*, while in *Raymondia* it varies greatly and is absent in one of the known species. The first anal vein of these genera is represented by a very short vestige in the basal part of the wings.

The wings of the American genera *Trichobius*, *Kesselia*, *Paratrichobius*, *Speiseria*, *Synthesiostrebla*, *Pseudostrebla*, *Eldunnia*, *Strebla* and *Euctenodes* have not the above-mentioned characters of the wings of the Old World genera. Their wings are remarkably uniform in their structure. They have six longitudinal and three cross-veins, except *Kesselia*, in which according to Curran (1934) the cross-veins are absent. Their sixth longitudinal vein represents the anal vein. All the veins are uniformly covered with setae, while the cells are bare and the alula is absent. As these characters are present in the wings of both the generalised and the specialised American genera, we may assume that the ancestors of the wingless American genus *Paradyschiria* and of the genera with reduced wings had the same type of wing as the above-mentioned winged genera.

This assumption is supported by a study of the wings of *Aspidoptera phyllostomatis*, *A. busckii* and *Pterellipsis aranea*. The wings of *P. aranea* are reduced to small elongated pads. Their venation is shown by darkly pigmented, narrow irregular ridges, from which arise very small erect setae. The examination of these ridges shows that the first ridge, which extends along the costal margin of the wing, is formed by the fusion of the costal vein with the first longitudinal vein (radius 1). The second, third and fourth ridges correspond to the second (radius 2+3), the third (radius 4+5) and the fourth (median 1+2) longitudinal veins respectively. The anterior cross-vein is represented by a broad fusion between the third and the fourth ridges. The ridge extending along the inner margin of the wing is the modified anal vein. The fusion of the dorsal part of this ridge with the fourth ridge is formed by the posterior cross-vein. The fifth longitudinal vein (cubitus 1) is represented by the short obliquely directed ridge between the anal vein and the middle part of the fourth longitudinal vein. The wings of *Aspidoptera phyllostomatis* and *A. busckii* are reduced to small oval pads. The wings have six ridges, which undoubtedly correspond to the six greatly modified longitudinal veins. In the other species of the genus

Aspidoptera and in the genus *Metelasmus* the number of ridges is indistinct. With the exception of *M. pseudopterus*, the reduction of the wings of the Streblidae did not correlate with the development of the short strong legs and the dorso-ventral flattening of the body, as it did in the other ectoparasitic Diptera Hippoboscidae.

The thorax of the most generalised genera *Nycteribosca* (Fig. 4 A) and *Raymondia* and the American genera *Trichobius*, *Paratrichobius* and *Speiseria* has practically the same structure as that of the Diptera Acalypterae. In the first three named genera it is rounded or more or less rectangular, whereas in the other two genera it is ovoid when viewed from above. The mesonotum of the thorax of these genera is distinctly subdivided into the prescutum (mesoprescutum), the scutum (mesoscutum) and the scutellum (mesoscutellum), and is about as broad as the ventral surface, which is formed by the sternopleura (*strn.*) and the pleurotrochantines (*pleu.*). The mesopleura (*mes.*) and the pteropleura (*pter.*) of the thorax occupy the lateral position. Of these sclerites the former are separated by a very narrow membrane from the prescutum, and the latter by the basal sclerites of the wings from the scutum; ventrally both sclerites are fused with the sternopleurum. The mesopostnotum (*mspn.*) of the genera *Nycteribosca* and *Raymondia* has the same structure as that of the highest Diptera; it lies below the scutellum. In the American genera *Trichobius*, *Paratrichobius*, *Speiseria*, *Eldunnia*, *Strebla* and *Euctenodes* this sclerite is very convex and projects posteriorly. The metapleura (*met.*) of these genera are also better developed than those of the Old World genera and, together with the mesopostnotum, form three very distinct projections in the posterior part of the thorax. The projecting parts of these sclerites are especially prominent in *Euctenodes*. The structure of the thorax of the genus *Aspidoptera* varies; in *A. busckii* it resembles that of the genus *Trichobius*, whereas in *Aspidoptera megastigma* it is as in the genus *Paradyschiria*. The thorax of *Paradyschiria* reveals an interesting case of convergence; its structure resembles that of the thorax of the Nycteribiidae. The sternopleura and the pleurotrochantines of this genus have become greatly broadened and flattened. Parallel with this modification, the mesopleura has been displaced dorsally and the scutum has become greatly reduced. It must be mentioned here that the mesopleura of the other dipterous parasites of bats, the Nycteribiidae, occupy the dorsal position. Moreover, their mesonotum has lost all the transverse sutures and therefore is not subdivided into the prescutum, the scutum and the scutellum. It forms together with the mesopostnotum an oblong plate which extends along the dorsal surface of the thorax from the anterior spiracles to the abdomen.

In the other American genus *Pterellipsis* the thorax is compressed, so that its pleural regions are flat. Its mesonotum, like that of *Paradyschiria*, is narrower than the sternopleura. The sternopleura of this genus are much longer than they are broad; their anterior part is produced into a flat, dorsally curved plate, which conceals the coxae of the fore-legs ventrally. The ventral surface

of the thorax of this genus bears a remarkable resemblance to that of the Nycteribiidae, especially its posterior part which is formed by the pleurotrochantines. The genus *Nycterophilia* (Ferris, 1916) deserves special consideration, because its thorax and abdomen are so strongly compressed that in general appearance the species of this genus resembles the Aphaniptera, especially when examined from above.

The structure of the thorax of the genera of the subfamily Streblinae differs from that of all the other American genera. It is rectangular when examined from above. Its anterior part is as broad as the posterior part of the head. The mesonotum of the generalised genera of this subfamily is slightly convex, whereas in the most specialised genus *Metelasmus* it is perfectly flat. The ventral surface of the thorax of all these genera is flat and is much narrower than the dorsal surface. This brief description of the thorax shows that this region of the body is uniform only in the subfamily Streblinae.

As regards the classification of the subfamilies, the chaetotaxy of the prescutum, scutum and the sclerites of the ventral surface of the thorax represents no distinctive character, but the arrangement and the number of the scutellar setae are of some importance.

In the Old World genus *Nycteribosca* the length of the scutellar setae and their number differ considerably. In the genus *Raymondia* there is a variable number of small setae on the scutellum, but its middle part always bears two strong setae in all the species. On the other hand, the chaetotaxy of the scutellum of the American genera differs from that of the Old World Streblidae. They all have four scutellar setae, except *Trichobius major*, which has eight and *Aspidoptera megastigma* and the genus *Paradyschiria* which have only two. In *Aspidoptera megastigma* and the *Paradyschiria* the scutellum has become greatly reduced and has lost the two setae. In the other species of the genus *Aspidoptera* the scutellum is normal and bears four setae. The disappearance of these setae through the reduction of the scutellum is also shown in the genus *Nycterophilia*. The scutellum of this genus is very small and has four setae, but the two anterior setae are so minute that they can only be seen under the high magnification of the microscope.

The legs are also not uniform in this family. In some genera their structure shows a distinct correlation with that of the thorax. In the generalised genera *Nycteribosca*, *Raymondia*, *Trichobius*, and *Kesselia* the legs are strong, the hind pairs being slightly longer than the other pairs. Those of the genera *Aspidoptera* and *Paradyschiria* are very stout, short and all about the same length. The legs of the latter genus deserve special mention. The femora of its fore-legs are greatly enlarged and each tibia, as in the Nycteribiidae, is marked by a slightly furrowed ring of much lighter pigmentation. The legs of *Speiseria*, *Paratrichobius*, *Synthesiostrebla*, *Pterellipsis* and *Strebla* resemble those of the Nycteribiidae, especially the hind pair. In the genus *Pterellipsis*, of which the ventral plate of the thorax (sternopleura + pleurotrochantines) is typically nicteribiid, the articulation of the legs is also nicteribiid. Moreover, the femora of the

middle and the hind-legs of this genus are partly ringed ventrally, not far from their base. The legs of *Paratrichobius* much resemble those of *Pterellipsis*; the femora of its middle and hind-legs are also partly ringed ventrally. The middle and the hind-legs of the five above-mentioned genera of the Streblidae and the legs of the Nycteribiidae show an interesting case of convergence. In both families one of the segments of the legs has become greatly elongated, attenuated and flexible, but it is not the same segment which has undergone this modification in these two families. In the mentioned genera of the Streblidae the tibiae have become long, attenuated and flexible, whereas in the Nycteribiidae the first tarsal segments have acquired this structure. In the genus *Nycterophilia* the coxae and the femora of the fore-legs have undergone parallel modification with the thorax. They are greatly flattened and are very broad, so that the fore-legs of this genus resemble more or less those of the Aphaniptera.

The abdomen of these Diptera is sacciform, with the exception of that of the genus *Nycterophilia*. It is almost entirely membranous as there are present only a few sclerotised parts: the first and the second sternites (Fig. 3 C, *st.* 1, *st.* 2), the lateral parts of the first + second tergites (*tr.* 1 + 2), and a few small plates with the apical cone in the abdomen of the female and the hypopygium in the abdomen of the male. Traces of segmentation are present only in the two species of the genus *Nycteribosca*, in *Trichobius major* and *Nycterophilia coxata*. The last-named species represents an exception, its sternites are partly sclerotised, and their chaetotaxy very much resembles that of the abdomen of the Aphaniptera. The development of the membranous wall of the abdomen in these Diptera is correlated with their breeding habit. They reproduce fully developed last-stage larvae—one at a time—which pupate immediately after extrusion from the uterus. The measurement of the last-stage larva of the different genera shows that it occupies a little more than four-fifths of the abdominal space. A larva of this size requires a considerable distension of the abdominal wall during its growth. This distension would be impossible without the transformation of the sclerotised tergites and the sternites into elastic membrane.

The dorsum of the abdomen and its chaetotaxy is correlated with the structure of the wings. In all the genera which have the normal or only slightly altered wings, the largest portion of the dorsum of the abdomen is bare and forms a shallow longitudinal groove. This groove is very distinct in the males and in the females if their abdomen is not distended by the developing larva. In all the Old World genera along each side of this groove there are present rows of very long, slightly decussate setae. The function of this groove and of the decussate setae was explained by Speiser (1900). According to this author the groove serves as a shelter for the interlocked wings at rest, while the long decussate setae, which bend obliquely across the wings, hold the latter in position and protect them during the progress of the parasites amongst the fur of the skin of the bats. The structure of these setae is different in the American winged genera; they are not so numerous as in the Old World genera and

are only a little longer than those of the ventral surface of the abdomen. In the wingless genus *Paradyschiria* and in the genera *Pterellipsis*, *Aspidoptera* and *Metelasmus* which have reduced wings, the dorsum of the abdomen is not grooved and bears setae of the same structure and arrangement as those on the other parts of the abdominal wall.

The abdomen of the females ends in an apical cone (*ap.c.*). The structure of this cone varies in the different species of the same genus. The abdomen of the males ends in a sclerotised, hemispherical hypopygium which is attached to the posterior end of the abdomen ventrally. In the antero-ventral part of the hypopygium there is a slit-like genital opening through which the two claspers (*cl.*) and the aedeagus (*aed.*) may protrude (Fig. 3 D and Fig. 4 D). Near the anterior edge of the genital opening of the Old World genera there are present two digitiform or ovoid processes (*dig.*) which generally bear two sensory setae. These processes are absent in all the American genera which have been examined. Inside the hypopygium the claspers are attached to a gutter-shaped sclerite which ends in an apodeme (*ap.*). The structure of both claspers is the same, with the exception of the genus *Raymondia*, where one of the claspers is very much smaller than the other. The aedeagus represents a long, thin-walled tube which lies in the guttered part of the sclerite of the claspers when at rest. As far as it is possible to see without dissection, the proximal end of the aedeagus of the Old World Streblidae is directly connected with its apodeme which is longer than that of the claspers. In the American Streblidae this apodeme is separated from the aedeagus by the framework of a very complicated structure, which apparently represents a part of the mechanism for the protraction of the aedeagus. Besides the difference in the structure of this part of the aedeagus and the absence of the digitiform processes, the American Streblidae have much thicker and shorter apodemes than those of the Old World Streblidae.

Before concluding this general description of these Diptera, it is necessary to consider the structure and systematic position of the genus *Ascodipteron*.

In 1896, Adensamer described the extraordinary female of an endoparasitic fly which he named *A. phyllorinae*. He studied the internal structure of this endoparasite and indicated its striking similarity to that of the Pupipara. However, he could not refer this insect to any family of this group, but believed that the male must be free-living on bats.

For this insect Monticelli (1898) created a new family Ascodipterinae. Muir (1912) studying males, which he bred from the puparia, found that structurally they do not differ very much from the species of the genus *Nycteribosca*. Therefore, he referred the genus to the Streblidae. According to Muir the life history of this insect is as follows: Males and females newly emerged from the puparia have fully developed wings and legs. With its enormously developed proboscis, the labella of which are armed with fourteen very strong, toothed blades, the female pierces the skin of the bat which it parasitises and drags itself into the wound. Here it becomes encysted after shedding its legs and wings. Immediately after encystment the abdomen of the female begins to

grow round the thorax and the head, so that in a comparatively short time it becomes flask-shaped. In the imbedded female only the posterior end of the abdomen protrudes through the opening in the skin of the bat. This endoparasitic female produces fully grown last-stage larvae which on ejection, one at a time, from the uterus, drop to the ground and immediately form the puparium. The male differs from the female in the structure of the body and especially of the head and the proboscis. The latter is very much smaller and resembles that of the genus *Nycteribosca*.

As regards the systematic position of this genus, it is impossible to accept the view of Monticelli. This author placed too much importance upon the highly specialised character which is presented only in the female. I quite agree with Muir's reference of these insects to the Streblidae. However, on account of the difference in the structure of the body, the venation of the wings and the unique structure of the adult female and its endoparasitic habit, the genus must be placed in a new subfamily Ascodipterinae.

On the basis of the morphology the Streblidae fall into the following four subfamilies: the Nycteriboscinae, the Ascodipterinae, the Trichobiinae and the Streblinae.

These subfamilies are easily separated by the following key:

1. Latero-vertices with the eyes not separated by membrane from the other regions of the head. Wings always present, with five longitudinal and two cross-veins (in *Ascodipteron* the anterior cross-vein indistinct and the posterior cross-vein absent); alula usually present. Scutellum with variable number of setae.
   Streblidae of the Old World **2**

   Latero-vertices with the eyes separated by membrane from the other regions of the head. Wings usually with six longitudinal and three cross-veins, or reduced to oblong or oval pads, or absent; alula absent. Scutellum with four scutellar setae, except in *Trichobius major*, *Aspidoptera megastigma* and the genus *Paradyschiria*.
   Streblidae of the New World **3**

2. Wings with well-developed five longitudinal and two cross-veins. Sexes do not differ structurally, except the genitalia. Subfamily **NYCTERIBOSCINAE**.

   Wings with five longitudinal veins, the fourth longitudinal vein thin and faint, the anterior cross-vein indistinct, the posterior cross-vein absent. Sexes differ greatly structurally: male free-living on bat; female endoparasitic, it sheds wings and legs before entering the skin of the bat, where it becomes encysted and flask-shaped.
   Subfamily **ASCODIPTERINAE**.

3. Head more or less rounded, especially in the posterior part, neither triangular nor trapezoidal, without ctenidium, its posterior part narrower than the thorax and elevated; palps foliaceous, rounded or somewhat pointed, directed always more or less dorsally. Thorax varying in structure, more or less rounded or ovoid, mesonotum convex or strongly convex, as broad as or narrower than the sternopleura.
   Subfamily **TRICHOBIINAE**.

   Head triangular or trapezoidal, its posterior part as broad as the anterior part of the thorax, usually with ctenidium; palps triangular, contiguous and horizontal, except in *Pseudostrebla* and *Eldunnia*. Thorax rectangular, the mesonotum slightly convex or flat, broader than the sternopleura.
   Subfamily **STREBLINAE**.

Finally, I should like to point out that the present systematic position of the Streblidae, as well as of all the other families of the group Pupipara, is not satisfactory. The inclusion of these flies together with Hippoboscidae, Nycteribiidae and Braulidae in this group was based upon an inadequate knowledge of their breeding habits and the superficial resemblance of their structure. As regards breeding habit, neither of these families is pupiparous, therefore, the terminology of the group is erroneous. The Hippoboscidae, the Streblidae and the Nycteribiidae are larviparous, whereas the only species of the family Braulidae, *Braula cæca* Nitz., which lives on hive bees, is oviparous. Its breeding habit was described correctly for the first time by Skaife (1921) and subsequently has been confirmed by several students of beekeeping.

The general resemblance in the structure of the members of these families is not a phylogenetic character. It is a specialised character and is due to the adaptation of these Diptera to almost identical environmental conditions; in other words, to the influence of similar mechanical factors in their microclimate. I have already stated in the introduction to this paper and elsewhere (Jobling, 1929) that in attempting to find the relationship of these flies to the other Diptera it is necessary to distinguish the most generalised structural characters from those produced by convergent evolution, since only the generalised characters give us a key to a correct interpretation.

That the group Pupipara is not a natural one was recognised by Müggenburg (1892) and subsequently by Speiser (1908), Massonat (1909), Muir (*loc. cit.*) and Falcoz (1926). The group Pupipara should be nullified. As the structure of the most generalised Hippoboscidae shows close affinity to the Muscidae, they must be placed at the end of the Calypterae, close to the family which have the genus *Glossina*.

The presence of the ptilinum in *Cyclopodia sykesi* and of its vestige in the genera *Nycteribosca* and *Trichobius* indicate that the Streblidae and the Nycteribiidae belong to the Schizophora. As regards the position of the Streblidae among the Schizophora, I think we have quite enough evidence for placing them among the family of the Acalypterae. The venation of the wings and the structure of the most generalised members of this family are typically Acalypterate.

The Nycteribiidae are much older geologically than the Streblidae, and they constitute the most peculiar and sharply defined family of the Diptera. Owing to their much longer association with bats, the Nycteribiidae have become structurally much more modified from the other Diptera than the Streblidae and have completely lost their wings. However, they have some common characters with the Streblidae, and must therefore be placed next to this family of the Acalypterae.

It is impossible to state definitely to which family of the Acalpyterae the Streblidae and the Nycteribiidae are closely related. Williston (1908) believed that there is a close relationship between the Streblidae and the Borboridae, while Falcoz (*loc. cit.*) connects the Streblidae and the Nycteribiidae with the Borboridae and the Helomyzidae respectively.

The general structure of the body of *Braula cœca* shows some resemblance to the Phoridae, but this resemblance reveals nothing more than convergence. The species has a well-developed ptilinum and therefore it must be referred to the Schizophora. There is no relationship between this genus and the Thaumatoxena of the Phoridae as stated by Börner (1908). The only part of the body of this fly which retains some generalised character is the proboscis. My own examination of the structure of the proboscis shows that its different parts, and especially the maxillae, resemble those of the proboscis of some Acalypterae. Therefore it should be also referred to this superfamily.

Genera of the subfamily Streblinae

According to the characters given in the preceding section, the subfamily Streblinae will include the following five genera: *Pseudostrebla*, *Eldunnia*, *Strebla*, *Euctenodes* and *Metalasmus*. The genus *Pseudostrebla* with a single species *P. ribeiroi* was described by Lima (1921). The description shows that it resembles *Strebla*. The head of this genus is trapezoidal and is as broad in its posterior part as the anterior margin of the thorax. The thorax is rectangular and its dorsal surface is broader than the ventral. As these characters are common to the other genera of the Streblinae, this genus, though lacking a ctenidium, should be referred to this subfamily.

The genus *Eldunnia* with a single species *E. breviceps* was described by Curran (1935). My own examination of the paratype of this species shows that it is interesting in many respects and in its structure related to *Pseudostrebla ribeiroi*. Its head is trapezoidal when examined dorsally, as broad in the posterior part as the anterior part of the thorax and provided with a ctenidium. The ctenidium of the specimen studied by me is composed of only nineteen spines and does not extend to the dorsal surface of the head as does that of the genera *Strebla*, *Euctenodes* and *Metelasmus*. The palps of this genus are much smaller than those of the three genera just mentioned. They are subrectangular with transverse apices and are directed more or less dorsally like the palps of the genus *Pseudostrebla*. The thorax of this genus is rectangular and broader dorsally than ventrally. It seems to me that morphologically *Eldunnia* represents a connecting link between *Pseudostrebla* and *Strebla*, and it is undoubtedly more closely related to the former genus.

The other three genera of this subfamily, *Strebla*, *Euctenodes* and *Metelasmus*, differ from the two described genera in many details. In all of them the posterior margin of the head, which is formed by the occiput and the postvertex, is very flat and overlaps the anterior part of the thorax. It is sharp-edged, festooned and bears strong setae which are directed posteriorly. The other portion of the dorsal surface is also flat and slopes anteriorly. All the regions of this surface are strongly sclerotised and clearly defined. The ctenidium of these genera extends to the dorsal surface and consists of numerous very darkly pigmented spines. The palps have already been described; they

represent the enlarged type of the palps found in the genus *Eldunnia*. The antero-lateral margin of the palps of the genus *Strebla* is festooned, while their ventral surface is evenly sclerotised and is broken only by the thecae of the setae which have the same structure and arrangement as those on the palps of the genus *Eldunnia*. In the genera *Euctenodes* and *Metelasmus* the festooned edge of the palps and their ventral surface are divided into separate irregularly shaped plates.

In all the genera of this subfamily, with the exception of *Pseudostrebla*, which I have not examined, the frontoclypeus (Fig. 3 A, *fc.*) has more or less the same structure and position. In the posterior concave parts of this sclerite there are present the antennae. Each antenna consists of two segments, the pedicle and the flagellum. The latter segment lies in the cavity of the pedicle and bears the branching arista. The general structure of the antennae of the Streblidae was given by me (Jobling, 1929), and those of the two genera of this subfamily, *Strebla* and *Euctenodes*, were very well figured by Kessel (1924). Here it is necessary to point out that the antennae of the genera *Eldunnia* and *Strebla* are much more exposed than those of the genera *Euctenodes* and *Metelasmus*. In these two genera they are not only concealed by the anterior edge of the laterovertices, but also by the flat projecting posterior edge of the frontoclypeus.

The eyes in this subfamily vary greatly. As regards the genus *Pseudostrebla*, it is impossible to state whether they are present or absent, since they are not mentioned in the original description. In the genus *Eldunnia* they are small, darkly pigmented and composed of an indistinct number of facets. In the genus *Strebla* they are seven-faceted, while in *Euctenodes* the number of the facets varies. In the genus *Metalasmus* each eye is reduced to a small ill-defined facet (Fig. 3 A, *e.*).

The only parts of the proboscis which are seen externally are the labium and the palps (Fig. 3 B). The labium is composed of the more or less bulbous theca (*th.*) and the long, narrow labella. The position of the labella varies with regard to the other parts of the head when at rest. In the genera *Pseudostrebla*, *Eldunnia*, *Strebla* and *Euctenodes* it lies between the palps, whereas in the genus *Metelasmus* it lies between the triangular postpalpal plates.

The theca lies in the sinuated part of the ventral surface of the head, between the postgenae. Its structure varies in the genera as follows: According to the original description, it is triangular in the genus *Pseudostrebla*, and it is also triangular in the genera *Eldunnia* and *Strebla*. The theca of the genus *Euctenodes* is bulbous, while that of the genus *Metalasmus* is octagonal (Fig. 3 B).

As regards the structure of the head of the genera *Strebla* and *Metalasmus*, it is necessary to mention here the presence of the special setae or the remiform scales (Fig. 3 A, *r.s.*). Each of these scales is situated behind the dorsal part of the ctenidium and is directed laterally. These scales are very broad in the genus *Strebla* and very narrow and curved in the genus *Metelasmus*.

The thorax of these genera differs in several details. The mesonotal suture is complete only in the genera *Pseudostrebla* and *Eldunnia*; in the other genera it is interrupted in the middle, so that the scutum is incompletely separated from the prescutum. The second suture described by Kessel (1924) in the genus *Euctenodes* represents only the narrow markings of the much darker pigmentations of the prescutum. The prescutum of the genus *Eldunnia* is twice as long as the scutum, and its anterior part has a triangular projection on each side of the median suture. The inner side of this projection is straight, the outer curves inwards forming an excavation. When the head is tightly applied to the thorax, each projected part of the prescutum becomes inserted into a corresponding depression of the posterior part of the head, while each postero-lateral part of the head is received by the excavated part of the thorax. The prescutum of the genera *Strebla* and *Euctenodes* is three times as long as the scutum. In the former genus its anterior border is slightly incurved between the parts of the pronotum, whereas in the latter it is curved slightly outwards. In all the genera of this subfamily, with the exception of the genus *Metelasmus*, the mesonotum is slightly convex. The mesonotum of the genus *Metelasmus* is perfectly flat and the prescutum is five times as long as the scutum. In this genus the anterior border of the prescutum is slightly incurved between the humeral calluses of the pronotum. Each pronotal part (*pr.*) is almost completely fused with the mesopleurum (*mes.*), and at this point slightly projects laterally. Between these projected parts the dorsal surface of the thorax is a little broader than in the posterior part. The scutellum of this genus is rhomboid, very small and separated from the scutum by a very narrow, clear membrane.

With the exception of the anterior part of the sternopleura, the ventral surface of the thorax presents no marked difference in the structure of this subfamily. The anterior part of each sternopleurum of the genus *Eldunnia* has a more or less conical projection with a rounded apex. The inner side of this projection is straight and together with that of the other projection forms a deep inflection in the anterior border of the ventral surface of the thorax. In the genus *Strebla* the projected part of each sternopleurum is much shorter and broader than in *Eldunnia*, so that the inflection of the anterior margin of the ventral surface of the thorax is very shallow. The anterior part of each sternopleurum of the genus *Euctenodes* very much resembles that of the genus *Metelasmus*; in both genera the anterior margin of the ventral surface of the thorax, between the apices of the projected parts of the sternopleura, is incurved. This incurvature is much narrower and deeper in the former genus.

As regards the chaetotaxy of the thorax, no distinctive character is present in all the genera of this subfamily. The prescutum and the scutum of the genus *Eldunnia* are uniformly covered with small setae, which are slightly longer on the anterior margin of the prescutum and in the most posterior row of the scutum. In the genera *Strebla*, *Euctenodes* and *Metelasmus* the setae of the prescutum and the scutum are not so numerous as in the genus *Eldunnia*, and they are also differently arranged. The genus *Euctenodes* differs from the other

genera of this subfamily in the presence of a curved row of closely situated and darkly pigmented short setae on each part of the pronotum, immediately behind the humeral callus. In their arrangement and structure these setae resemble more or less an imperfectly developed ctenidium. The arrangement of the setae on the mesopleura (*mes.*) are the same in all these genera, but the length of these setae is different. These setae are only a little longer than those of the prescutum in the genus *Eldunnia*, whereas in the genera *Strebla*, *Euctenodes* and *Metelasmus* they are definitely very long. One of these setae which arises in the middle of the posterior half of each mesopleurum is the longest and is directed much more laterally than the others. The ventral surface of the thorax of the genus *Eldunnia* bears numerous small setae. In the genus *Strebla* these setae are very thin, and the lateral part of each sternopleura is bare. The arrangement of the setae on the ventral surface of the thorax of the genus *Euctenodes* is as in *Eldunnia*, but the setae are stronger. The genus *Metelasmus* differs from the other genera in the presence of a few stronger setae in the most anterior part of the sternopleura near the median suture.

With the exception of the genus *Metelasmus*, the wings are normally developed in this subfamily. Their structure is the same in the genera *Pseudostrebla*, *Eldunnia* and *Euctenodes*, but in the genus *Strebla* they are pointed at the apex and are much narrower than those of the three genera just mentioned.

The legs also vary in structure in this subfamily. They are very stout in the genera *Euctenodes* and *Metelasmus*, while in the genus *Eldunnia* and especially in the genus *Strebla* they are slender. The posterior legs of the last-named genus are greatly elongated, they are twice as long as the fore-legs. Moreover, the first tarsal segment of all the legs of this genus is as long as the three following segments together. According to the original description the legs of the genus *Pseudostrebla* are as in *Strebla*, but relatively more robust. In the genus *Euctenodes* the first tarsal segment of the hind-legs is as long as the following three segments together, while in the genera *Eldunnia* and *Metelasmus* the first tarsal segment of the hind-legs is only as long as the two following segments together. The coxa of the fore-legs of the genus *Eldunnia* occupies the ventral position and is practically invisible from above. By this character the genus differs from the other genera of this subfamily, since in all of them the convex flap-like part of the coxa of the fore-legs projects between the head and the thorax dorsally, and its marginal setae extend over the dorsal surface of the thorax.

The structure of the abdomen of the genus *Metelasmus* is different. It has already been shown that the dorsal surface of the abdomen of this genus bears setae, whereas in the other genera of this subfamily, which have normal wings, this surface is bare. There is also a slight difference in the structure of the first + second tergite and the first and the second sternites. However, this difference I cannot yet consider as a generic character, because I have not studied both sexes of all these genera. Furthermore, the structure of these parts of the abdomen varies very much in the species of the genera of the other subfamilies.

The genera of this subfamily can be very easily separated by the following key:

1. Ctenidium absent.

Pseudostrebla Lima, 1921.
With a single species *P. ribeiroi* Lima, 1921.

Ctenidium present 2

2. Ctenidium composed of eighteen or nineteen spines does not extend to the dorsal surface of the head; postero-lateral parts of the dorsal surface of the head slightly convex. Palps, together narrower than the anterior margin of the head, project antero-dorsally. Mesonotal suture complete.

Eldunnia Curran, 1935.
With a single species *E. breviceps* Curran, 1935.

Ctenidium composed of numerous spines and extending to the dorsal surface of the head; posterior part of the dorsal surface of the head flat, its edge festooned and overlapping the anterior part of the thorax. Palps large, horizontal, as broad together in their posterior part as the anterior margin of the head, their outer edge bent dorsally and festooned. Mesonotal suture incomplete 3

3. Festooned edge and ventral surface of palps not divided into sclerotised plates; posterior edge of the frontoclypeus not overlapping the antennae dorsally. Posterior legs greatly elongated, twice as long as the frontal pair.

Strebla Wiedeman, 1824.
With two species: *S. vespertilionis* Fabritius 1805 and another doubtful one *S. avium* Macquart, 1854.

Festooned edge and ventral surface of the palps divided into sclerotised plates; posterior edge of the frontoclypeus flat, short and overlapping the antennae dorsally. Posterior legs not elongated 4

4. Eyes faceted. Mesonotum slightly convex, prescutum three times as long as scutum. Wings normal. Dorsal surface of abdomen bare.

Euctenodes Waterhouse, 1879.
With two species: *E. mirabilis* Waterhouse 1879 and *E. tonatiae* Kessel, 1924.

Eyes reduced to single not quite distinct facet. Mesonotum flat, prescutum five times as long as scutum. Wings reduced to oval pads. Dorsal surface of the abdomen bearing setae.

Metelasmus Coquillett, 1907.
With a single species *M. pseudopterus* Coquillett, 1907.

Redescription of *Metelasmus pseudopterus* Coquillett (1907)

(Figs. 2 and 3)

The genus *Metelasmus*, with a single species *M. pseudopterus*, was erected by Coquillett (1907) for a single specimen collected in Paraguay, South America, on a bat, *Artibius literatus*. The author did not compare his new genus with the other closely related genera *Strebla* and *Euctenodes*, which were already known, and owing to the condition of his specimen he could not see the antennae and the eyes. In the specific description of this streblid he used only one morphological character—the length of the specimen—while the rest of the description was based on the colour of the different parts of the body. It is only the genus

Nycteribosca in which the colour of the head can be used as a character for the subdivision of the species; in all the other members of this family the colour has no specific value, because it may vary from straw yellow to dark brown in the same species according to the age and the condition of the specimen.

This redescription is based upon the examination of the two specimens, both males, from the collection of Major L. H. Dunn. These specimens were found

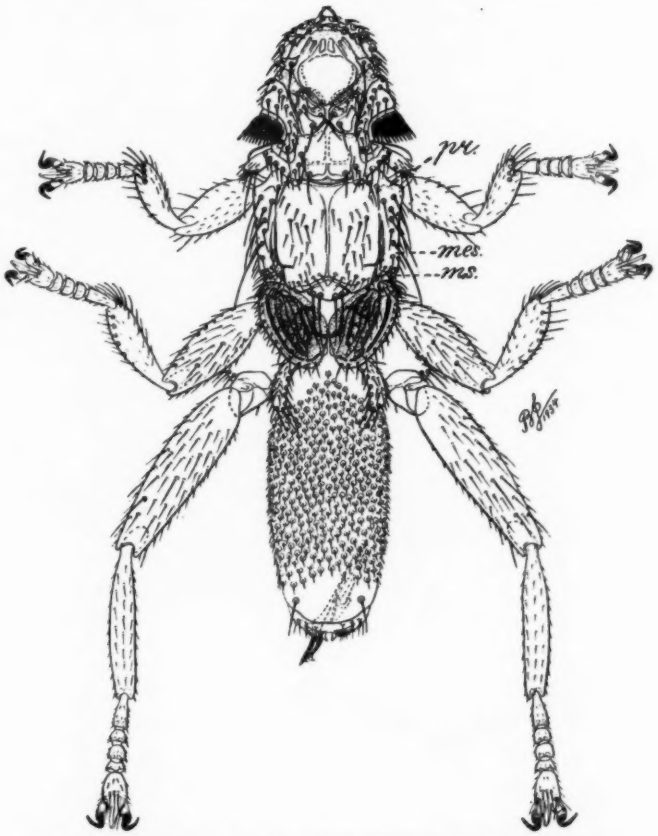

Fig. 2. *Metelasmus pseudopterus* Coquillett. Dorsal view of male.

on a bat, *Artibius jamaicensis jamaicensis* Leach, in Puente Natural, Panama. As far as I know this is only the second record of this species.

Body 2·1 mm. long (on slide). Head, including the palps, bell-shaped when examined from above; frontoclypeus (*fc.*) bare, its posterior margin broadly pointed, sharp-edged and overlapping the antennae; anteriorly to the frontoclypeus there are present two sclerotised plates irregular in outline, laterally to each of them and partly concealed under the antero-lateral border of the

frontoclypeus there is a much larger, more or less oblong sclerotised plate; laterovertices (*lv.*) more or less triangular, with thickened edges; each of them bears five very small darkly pigmented setae in the anterior part, four much stronger setae in the middle and two strong setae close to the postero-inner margin. One-faceted eyes (*e.*) present; they lie in the sinuated lateral part of the

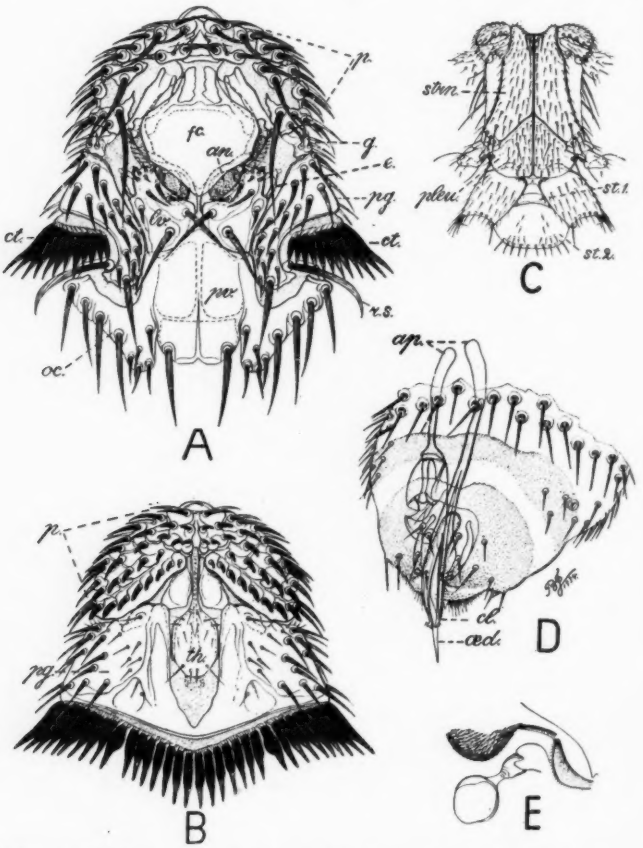

Fig. 3. *Metclasmus pseudopterus* Coquillett. A, dorsal, and B, ventral views of head, × 100; C, ventral view of thorax, × 33; D, ventral view of posterior end of abdomen of male, × 100; E, halter, × 340.

laterovertices. Postvertex (*pv.*) flat, its anterior triangular part projecting between the laterovertices and partly overlapping their posterior part, its posterior third greatly flattened and overlapping the anterior part of the thorax; it bears two strong setae. Each dorsal part of the occiput (*oc.*) V-shaped, flat and overlapping the front coxae and the anterior part of the thorax; it bears five small setae in the posterior part and four strong setae along

the festooned lateral edge. Dorsal part of each postgena (*pg.*) hook-shaped, separated from the dorsal part of the ctenidium and the occiput by a broad membrane; its surface bears two rows of small setae and one strong seta close to the posterior end. In the sinuated part of each postgena, behind the ctenidium, there is a large theca from which arises a long, distally curved remiform scale (*r.s.*). Genae (*g.*) very small, irregularly shaped sclerites, with a few small setae. The ventral surface of the head flat and almost entirely formed by the postgenae (*pg.*); the surface of each postgena bears a few very fine setae in the middle and about an equal number of very much stronger setae laterally. Theca (*th.*) of the labium octagonal with a few very fine, small setae in the anterior half, two setae of the same size near the posterior border and one much longer seta in each postero-lateral part; labella longer than the theca, it lies between the triangular plates which correspond to the post palpal plates of the other Streblidae. Palps (*p.*) strongly concave ventrally their dorsally bent lateral border composed of separated, irregularly shaped plates; the most posterior triangular plate is the largest, it bears three small and one very long, strong seta directed posteriorly; the next plate rounded with one seta and followed by another triangular plate bearing three setae, of which the posterior seta is the strongest; in front of this plate there are two small plates each with one seta; mesally to this plate, in front of the frontoclypeus there is an **8**-shaped plate with a very small seta in the middle and two strong setae pointing in opposite directions. In front of this plate there are two double plates bearing each two setae; the apex of each palp ends in a small, slightly flattened scale-like seta directed towards that of the other palp. The posterior border of the ventral surface is formed by a very thick, sclerotised and partly subdivided oblong plate, each subdivision of which bears a very stout, darkly pigmented tooth-like seta; there are three more rows of these setae, each seta arising from an irregularly shaped plate incompletely separated from the next. These setae, small near the inner border, but gradually becoming stronger towards the postero-lateral part of the palp, also become more pointed towards the most anterior row. The antero-lateral edge bears setae of the same structure as those of the dorsal plates of the palp. Antennae almost completely concealed inside the antennal pits formed by the posterior part of the frontoclypeus and the anterior part of the lateravertices; first antennal segment (pedicle) ovoid, its dorsal surface covered with very minute scales and bearing one very small, darkly pigmented seta; second segment (flagellum), consisting, in fact, of two segments: one globular (as far as it is possible to see without dissection) which lies inside the pedicle and the other the arista; the latter projects through the opening of the pedicle and lies laterally to the frontoclypeus, its branching tip almost reaches the dorsal triangular plate of the palp.

Thorax rectangular when examined from above, slightly broader in the anterior than in the posterior part; mesonotum flat; prescutum as long as broad and incompletely separated from the scutum by the mesonotal suture; the anterior and the middle of the posterior part of its surface bare, the rest bearing

a few small and three long setae; scutum very short, bearing a row of small setae along its posterior border and a very few small setae in the middle; scutellum small, rhomboid with four moderately strong setae arising very close to the posterior border; it is separated from the scutum by a very narrow membrane; mesopleura flat, as long as the prescutum, with a few short and a few very long setae; pteropleura very small and partly concealed by the wings. The ventral surface of the thorax flat; sternopleura (*strn.*) moderately setose, the setae very small in the anterior part and becoming stronger towards the posterior part; there are a few stronger setae in the most anterior part of the sternopleura, on each side of the median suture; the pleurotrochantines bear small setae, besides which each of them has one much stronger seta in the middle and two similar ones in the lateral corner. Legs strong, hind pair slightly elongated; coxae of the fore-legs strongly convex anteriorly, and projecting laterally as far as the most lateral spines of the ctenidium; dorsal surface of the femora of the fore-legs with a few small setae, the same surface of the femora of the other legs with many much stronger setae; tibia of the fore- and middle legs slightly dilated, with a few very small setae, a row of much stronger setae in the distal part and another row of similar setae along the outer surface; tibia of the hind-legs not dilated, with rows of very small setae.

Wings reduced to oval pads, 351 μ long; costal margin bearing many setae, the surface bearing very few setae; venation reduced to darkly pigmented ridges; halteres present, their structure is shown in the drawing (Fig. 3 E).

Abdomen twice as long as the mesonotum; the lateral parts of the tergite one + two, more or less strongly sclerotised and bearing strong, darkly pigmented setae along their edges; the first sternite (*st.* 1) narrow and curved, the second sternite (*st.* 2) almost as broad as long, with a few small setae in the middle and stronger setae along the posterior margin; abdominal wall bearing very many small setae, each arising from a large theca; the most posterior part of the dorsal surface bare, with two long lateral setae. Hypopygium cup-shaped (Fig. 3 D), attached ventrally; genital opening slit-shaped, its edge bearing tiny scales. On each side of the genital armature, under the flap of the genital opening there is a narrow curved plate, this plate bears three small setae on its posterior end and together with the plate of the opposite side forms a lyre-shaped pattern. Aedeagus (*aed.*) narrowly pointed, its proximal end attached to a rather complicated sclerotised structure which ends in a short, spatulated apodeme (*ap.*); two hook-shaped and toothed claspers (*cl.*) lie ventrally to the aedeagus being attached to the other slightly longer apodeme.

Nycteribosca bequaerti sp.n. (Fig. 4)

One type specimen, male, and three paratypes, two females and one male were examined from the collection of Dr J. Bequaert. This is the only species with a darkly pigmented head, recorded from Africa. Of all the species which

have the darkly pigmented head, it resembles very much *N. gigantea*; from this, however, it can be very easily separated by its larger size, the short labella of the labium, the presence of many very long setae on the dorsum of the abdomen, and also by the curvature of the basal part of the first longitudinal vein and the structure of the first basal cell, which is not enlarged in its anterior part.

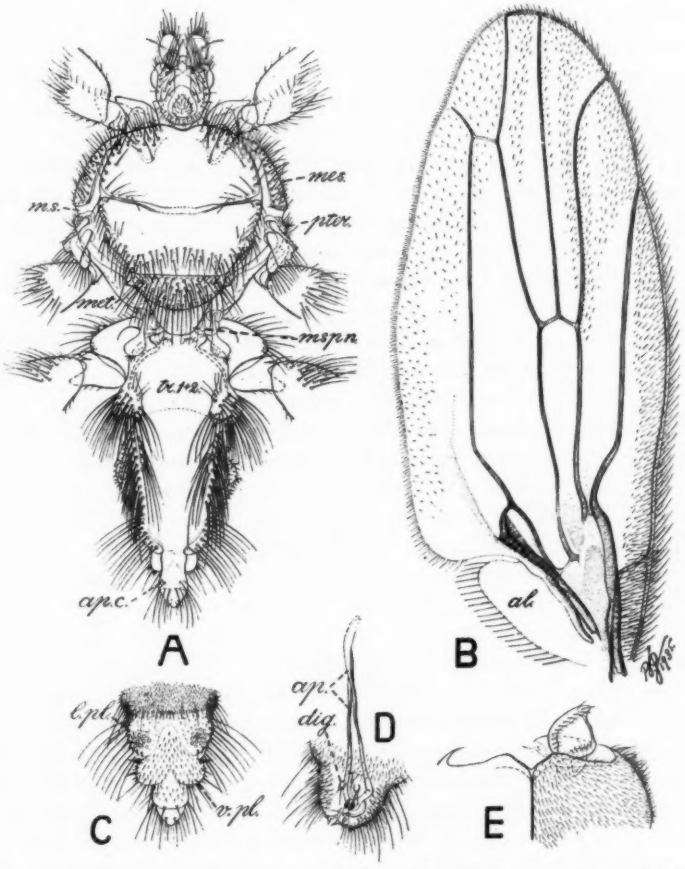

Fig. 4. *Nycteribosca bequaerti* sp.n. A, dorsal view of female; B, wing; C, ventral view of posterior end of abdomen of female; D, same of male; E, sternopleura.

In the venation of the wings and the chaetotaxy of the sternopleurae the new species resembles *N. nigriceps* (Jobling 1934), but from this species it can be separated by its size, the different shape of the head, and the thorax, and also by the very different chaetotaxy of the prescutum and the scutum.

Body 4·5 mm. long (on slide). Head dark, distinctly more pigmented than the body; the lateravertices, besides four long setae arising from their anterior

border, bear short setae; mediovertex very broad; postvertex piriform or rhomboid, with many very small setae, many fine setae on the occiput behind the postvertex; postgenae bearing long setae along their edge and short setae behind. Eyes large, very distinct. Palps oval. Labella of the labium very short; theca large, bell-shaped.

Thorax rounded, broader than long; prescutum with long erect setae in its antero-lateral parts and a row of the same setae along the lateral margins and near the lateral part of the mesonotal suture; scutum with long setae along its lateral margins and a transverse patch of the same setae in the posterior part; scutellum broadly rounded, with many long erect setae; the anterior part of the mesopleura with setae of the same length as those on the prescutum and much shorter setae ventrally; pteropleura with short setae. The sternopleura and the pleurotrochantines bear many very small setae (Fig. 4 E). Legs very strong; dorsal surface of femora with many strong setae, the ventral surface with very small setae; tibiae more thickly beset with small setae.

Wings 4·6 mm. long. One-fifth of the first longitudinal vein forms a bend in its basal part, the rest of this vein runs nearly straight; the second longitudinal vein bends more or less strongly towards the costal margin in its distal part; the third and fourth longitudinal veins are almost parallel near the apex of the wing; the fifth longitudinal vein has a sharp bend in the basal part; between this bend and the posterior cross vein it is straight; the anterior half of the first basal cell is straight; the costal, marginal and submarginal cells bear many setulae, they are much longer in the basal part of the costal and humeral cells and become gradually shorter towards the apex of the wing. Alula broadly rounded. Halteres presenting no distinctive character.

Abdomen with very many long setae on each side of the bare part of the dorsal surface; ventrally to these there are spine-like small setae; middle part of the ventral surface of the abdomen bears numerous very small setae and four very thin long setae near the posterior margin; lateral parts of the tergites one + two and the lateral plates (*l.pl.*) with many very long setae; ventral plate (*v.pl.*) cordiform, with very small setae and strong spine-like setae on its lateral borders. Between the setous middle part and the ventral plate the surface bears a very few tiny setae and a patch of pigmented discs, the lateral edges of this part have spine-like setae. The patch of pigmented discs is also present on each lateral part of the setous ventral surface of the abdomen. Apical cone of the abdomen large (*ap.c.*), its structure shown in the drawings A and C, Fig. 4.

The male abdomen differs from that of the female in the following respects: its posterior part has no plates, it ends in a hemispherical hypopygium of which the structure is shown in the drawing D, Fig. 4.

The specimens of this species were collected from a bat *Rousettus leachi* Smith, Bagilo, Uluguru Mountains, Tanganyika Territory.

RAYMONDIA INTERMEDIA sp.n. (Fig. 5)

One type specimen, female, was examined from the collection of Dr J. Bequaert. As regards the general structure of this new species, it can be placed between *R. planiceps* (Jobling, 1930) and *R. waterstoni* (Jobling, 1931). From

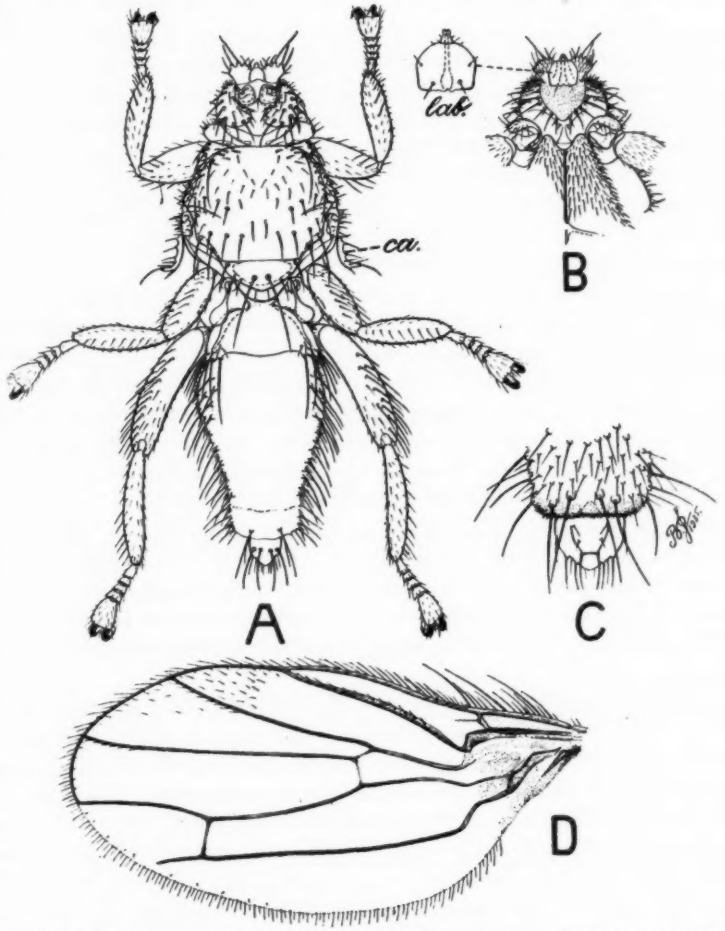

Fig. 5. *Raymondia intermedia* sp.n. A, dorsal view of female; B, ventral view of head and sternopleura; C, ventral view of posterior end of abdomen of female; D, wing.

the former species it can easily be separated by the structure of its head, which is narrower than the anterior part of the thorax and also by the absence of the terminal seta of the alula. In these characters it is related to *R. waterstoni*, from which, however, it differs in the structure and chaetotaxy of the thorax;

its mesonotum is not dome-shaped and is not broader than long as it is in the species just mentioned. Moreover, it differs from that species in the presence of the long setae on each side of the bare dorsal surface of the abdomen and, finally, in the form of the apical cone of the abdomen, of which the posterior border is not rounded.

Body 2 mm. (on slide). Head more or less rounded when examined from above, narrower than the anterior part of the thorax; mediovertex distinct, pale; each laterovertex thickened along the antero-inner border on which and on its lateral and posterior part it bears a few small setae; postvertex indistinct; postgenae bearing moderately strong setae along their inner margin and a few very small setae in the posterior part, along the edge of the concave part of the ventral surface of the head (Fig. 5 B). There is also one moderately strong seta in the lateral part of each postgena. Theca of the labium (*lab.*) slightly broader than long, with two very fine setae near its posterior margin. Labella very small. Palps presenting no distinctive character.

Thorax as long as broad; humeral processes moderately large, with two long and a few small setae; prescutum with two very long posthumeral setae, its surface not very setose and having a triangular bare space in the middle of its anterior part; scutum with a row of about eight setae across the middle and two small setae fairly close together in the middle of its anterior part; scutellum triangular, with the usual two very strong setae and a few small setae arising from its posterior border. Sternopleura and pleurotrochantines beset with small setae. Legs presenting no distinctive character.

Wings 2 mm. long. Alula vestigial, without terminal seta; humeral vein with one seta; no setulae in the costal cell; a batch of setulae in the distal part of the marginal cell and a few setulae in the first submarginal cell; third and fourth longitudinal veins divergent near the apex of the wing; fifth longitudinal vein having a zig-zag bend in its basal part. Halteres presenting no distinctive character.

Abdomen with strong, long setae on each side of its bare dorsal surface; its ventral surface bearing numerous fine setae which gradually merge into very minute and far more numerous setae laterally, especially in the antero-lateral parts of the abdomen. There are four long setae arising from the large thecae near the posterior border of the ventral surface and two setae of the same structure on the dorsal surface in front of the apical cone of the abdomen. Apical cone of the abdomen as long as broad, flattened and more or less triangular in its posterior part, with four dorsal and two ventral moderately long setae, besides four very small setae on its ventral surface, one much longer in each lateral part, and several on the posterior border (Fig. 5 A and C). The specimen was found on a bat, *Rhinolophus hildebrandii* K. And., in Rutschurie, Belgian Congo.

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Lettering

aed. aedeagus; *al.* alula; *an.* antenna; *ap.* apodeme; *ap. c.* apical cone of abdomen of female; *ca.* calipteron; *cl.* clasper; *ct.* ctenidium; *dig.* digitiform process; *e.* eye; *fc.* frontoclypeus; *g.* gena; *lab.* labium; *l.pl.* lateral plate of abdomen; *lv.* laterovertex; *m.* membrane; *mes.* mesopleurum; *met.* metapleurum; *ms.* mesopleural suture; *mspn.* mesopostnotum; *mv.* mediovertex; *oc.* occiput; *p.* palp; *pg.* postgena; *pleu.* pleurotrochantin; *pr.* part of pronotum; *pter.* pteropleurum; *pv.* postvertex; *r.m.* rostrum membrane; *r.s.* remiform scale; *strn.* sternopleurum; *st.* 1, first sternite; *st.* 2, second sternite; *tr.* 1+2, tergite 1+2; *th.* theca of labium; *v.pl.* ventral plate of abdomen.

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STUDIES OF THE EFFECT ON *T. GAMBIENSE* AND *T. RHODESIENSE* OF PROLONGED MAINTENANCE IN MAMMALS OTHER THAN MAN; WITH SPECIAL REFERENCE TO THE POWER OF THESE TRYPANOSOMES TO INFECT MAN. I.

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I. General introduction

The main theme of this series of papers will be the power of the three well-known members of the *brucei* group of trypanosomes, *T. brucei*, *T. rhodesiense* and *T. gambiense*, to infect man. Inextricably involved with this problem are two others, both of practical importance: (1) Can the African big game serve as a reservoir for human trypanosomiasis? and (2) What are the affinities existing among these three trypanosomes? This may appear to be an inversion of the real order of importance, but a study of the literature on the *brucei* group, past and recent, reveals that infectivity to man dominates all other considerations.

Within this group are placed certain important agents of human disease, whereas among the other so-called pathogenic trypanosomes there are none that trouble man directly. It is undoubtedly the desire to eradicate sleeping sickness that has supplied the incentive to the enormous amount of work done upon the polymorphic mammalian trypanosomes.

To a biologist with a completely unbiased outlook, if such a one exists, the infectivity of a trypanosome to man possesses no more importance than its infectivity to any other species of mammal. It is easy to forget that we have never been able to experiment upon man with anything like the same freedom that we can upon animals. *T. vivax* and *T. congolense* have been induced in the laboratory to infect hosts in which in nature they are unable to survive, and in all probability similar treatment applied on the same scale to *T. brucei* and

man would produce similar results. Where, however, a trypanosome has come to depend on man as its chief or sole mammalian host in nature, as seems to be true of *T. gambiense*, and has in consequence developed invariable pathogenicity to this highly resistant host, this quality does perhaps deserve some attention from the systematist. At the present time little is known about the relation of this group of trypanosomes to man. The papers of this series will contain some new observations on this subject, which may reduce or on the other hand may add to the existing confusion.

In these papers an attempt will be made to summarise our knowledge and particularly to state as definitely as possible the position reached by the investigations carried out at this Institute. For years past papers have issued from this laboratory dealing with various aspects of the biology of the *brucei* trypanosomes. A striking result of these publications has been their failure to stimulate anyone to a serious attempt at repetition of the investigations under different conditions of experiment. The simplest explanation of this failure is that the findings have been badly presented, and a final attempt is therefore now made to rectify this by simplification and compression.

The following subjects will be considered, though not necessarily in this order: *T. gambiense* in ruminant game; the effect of prolonged maintenance away from man on the infectivity of *T. gambiense* for man; similarly with *T. rhodesiense*; the infectivity of "wild" *T. brucei* for man; some observations on Bevan's "Kahondera" strain from Rhodesia; and lastly a brief summary of the position as it appears to the writer.

The relation of the polymorphic trypanosomes to man in nature is a problem that presents many difficulties, not least among them being the need for a liberal supply of volunteers. It has been possible of late in Uganda to meet this requirement, with the result that several interesting facts have come to light.

To appreciate the complex nature of the investigation it must be realised that individual men differ in their reactions towards a given strain of trypanosomes, and that individual strains differ in their power of using man; also that various incalculable factors exert an influence on the infectivity of a trypanosome—for example, prolonged passage in guinea-pigs (Duke, 1935).

In appraising the experiments described in the papers that follow, the reader must accept the assurance that the question of control has been kept constantly in view. The matter has already been discussed elsewhere (Duke 1934*a*). In the monkey park three sensitive *Cercopithecus* monkeys are kept as permanent controls, year in year out, under daily observation by examination of stained thick blood films. A stained thick blood film from every new monkey is taken daily for at least a week before the animal is used: no infection with a pathogenic trypanosome has ever been found among these new arrivals. Lastly, five "clean" young sheep, which wander about the laboratory precincts all day long, are examined in the same manner and, so far, with the same result. During the last two years only two monkeys carrying *T. brucei* (both experimentally

infected) have been present in the monkey park, and these for only a short period. No case of "casual" infection has ever been detected among the monkeys or guinea-pigs. All these measures of control are the result not of suspicion but of a lively appreciation of the importance of detecting either the accidental introduction of *T. brucei* from outside sources, or cross-infections in the monkey park itself. In every instance where a monkey has become infected in the course of fly transmission experiments a gland-infected fly has been demonstrated by dissection among those that fed upon the animal. The significance of this statement will be evident to anyone who has undertaken work of this kind.

And now for one or two general remarks about the technique employed. First of all, the method of infection. Whenever possible, cyclically infected tsetse are used—*G. palpalis* unless otherwise stated. As it is the normal method operating in nature, cyclical infection appears to me superior to syringe inoculation of trypanosomes in the peripheral circulation.

As an alternative, inoculation of one of the glands of an infective fly is used, the other being inoculated into a control animal. If infection results it is then due to the metacyclic forms of the trypanosome.

There are two important points to be observed when inoculating the glands of *Glossina*. A control injection into a susceptible animal is essential, and for this a portion or the whole of one gland will serve. Secondly, it is very helpful to use a transparent syringe so that the expulsion of the gland can be verified. The organ is apt to stick to the syringe walls, and it is sometimes necessary to recharge the barrel two or three times with fresh saline before the gland at length disappears. As a final precaution I fill the syringe once again with saline, expel the contents into a watch-glass, and examine. I find these measures absolutely necessary.

The injection of glands is not invariably successful, and failures must be disregarded unless the control animal becomes infected. But as a general rule this method, certainly when the fly is killed a few minutes before dissection, is as effective as fly-bite.

The men employed in these experiments are members of a band of some seventy native volunteers whose acquiescence made these investigations possible (Duke, 1934*a*). They all received a monetary reward, but this does not cancel their claim to our admiration and gratitude. Apart from the temporary inconvenience of a body temperature of 105 or 106° F., there are certain powerful ethical or sentimental objections which these men have to face before they can offer themselves. Some of them have now been infected twice, and a few three times.

Without the co-operation of natives there is little chance of solving the outstanding problems of sleeping sickness, and it is pleasant to record that the Uganda Government has acceded to my request to grant all these volunteers, in consideration of services already rendered, exemption from a year's taxes.

II. *T. GAMBIENSE* IN RUMINANT GAME

(1) *Review of published investigations on the behaviour of* T. gambiense *in ruminants*

The first investigators to study the behaviour in ruminants of strains of *T. gambiense* freshly isolated from man were Kleine and his colleagues in German East Africa, and the members of the second Royal Society's Commission in Uganda.

Kleine's important discovery of the cycle of development of the *brucei* group in *Glossina* opened up new lines of investigation. The German investigators studied the behaviour of *T. gambiense* in domestic ruminants, and found that though generally infective to sheep and goats this trypanosome tended to die out rapidly. The longest duration of infectivity to tsetse in any of these animals was 119 days (Kleine & Fischer, 1911).

Bruce and his colleagues of the Royal Society's Commission were the first to investigate the role of antelope as a reservoir of *T. gambiense* (Bruce *et al.* 1911). The Commission used three bushbuck, six reedbuck, and one waterbuck. The animals were captured by native hunters and brought to the laboratory at Mpumu. There is no record in the Commission's reports of the district where these animals were captured, but on my arrival at Mpumu in 1910 it was generally understood that they came from the tsetse-free game districts near Masaka or to the north-west of Bombo, about 90 miles west and 40 miles north-west of Kampala respectively. It is therefore unlikely that they were infected before their arrival.

Before infecting the animals at the laboratory a preliminary inoculation of each antelope's blood was made into a clean monkey in the case of all save one reedbuck and one bushbuck. None of these control monkeys became infected. The antelope were then all exposed to infection with what is definitely stated to be "*a human strain of T. gambiense*" by means of the bite of *G. palpalis* infected at the laboratory. In every animal, after an interval of from 11–25 days, trypanosomes were demonstrated in the blood either by direct observation or by subinoculation into a clean monkey.

It has been suggested that these experiments are valueless because the steps taken to exclude natural infections of *T. brucei* in the newly arrived antelope were inadequate. It is true that a single control injection of blood is not an exhaustive test, as will be seen when *T. rhodesiense* comes under discussion. But since these animals were all subjected to almost daily blood examination immediately after their exposure at the laboratory, and trypanosomes appeared in their blood just when they should have appeared in relation to that exposure, the evidence is almost conclusive that the sequence of events was indeed that claimed by the authors. An interesting feature is the large number of trypanosomes seen in the blood of some of these antelope—a phenomenon unusual in infections of ruminants with *T. gambiense*. But it must be remembered that

the strains used by the Commission were isolated during a big epidemic and so were probably different from the typical chronic *T. gambiense* with which subsequent Uganda investigations have been carried out.

The Commission regarded what I have more recently called the "lake shore" or "Damba trypanosome" as *T. gambiense*. This may or may not have been correct, but it was certainly never proven. If, then, they had used a strain derived from wild lake-shore fly or antelope their experiments would possess little or no significance. But in each case the use of italics makes it unmistakably clear that a human strain of *T. gambiense* was used to infect the antelope.

The point is of some importance because the subsequent history of these animals is the only recorded evidence that antelope may act as a reservoir of *T. gambiense*. Unfortunately, during the experiments on these antelope no tests were performed on man himself, so that the only result of these later investigations was the determination of the duration of survival of the trypanosome in the blood. This was recognised in the following sentence: "The point of practical importance to be established is whether these trypanosomes are pathogenic to man and this is obviously difficult to prove." At that time the difficulty of an appeal to man was much greater than it is to-day.

The conclusions eventually reached at the time of the closure of the Mpumu laboratory in 1912 were as follows:

"1. That the antelope may remain capable of infecting *G. palpalis* with *T. gambiense* for a period of at least 22 months after their original infection with this trypanosome.

"2. That there is some evidence to show that an antelope which has ceased to be infective for *T. gambiense* acquires some degree of immunity against re-infection." (Duke, 1912.)

Let us turn now to the work done by the League of Nations Commission on Sleeping Sickness in Uganda in 1926–7. A number of strains of *T. gambiense* from various parts of Eastern and Central Africa were studied by this Commission (Duke, 1928). No experiments were made with game animals but nine different strains were tested in sheep or goats or in both. Some of the lines of investigation started by the Commission were continued at this Institute. It was found that "Natural immunity against *T. gambiense*, as exhibited by sheep and goats, varies from complete immunity to a degree of susceptibility in which the trypanosome may be a contributory or even possibly the direct cause of death. This last contingency is, however, beyond doubt exceedingly rare. Between these extremes we find a peculiar partial immunity which operates solely on the transmissibility of the trypanosome by tsetse, abolishing this property by leaving the strain still capable of producing gut infections in tsetse and still demonstrable in the peripheral blood." "Immunity can be acquired by sheep and goats against *T. gambiense*. The earliest manifestation of the development of acquired immunity is the disappearance of the transmissibility of a strain by tsetse." (Duke, 1928 *a*).

It was also found that a strain of *T. gambiense* could retain in a sheep its transmissibility by *G. palpalis* for at least 15 months (Duke, 1931).

These investigations also revealed great differences between different strains of *T. gambiense* in their behaviour towards sheep and goats and in their ability to develop in tsetse, a point to be borne in mind in considering the evidence given below.

In addition to the laboratory experiments recapitulated above, Wenyon (1926) gives a list of instances of the recovery from African game animals of trypanosomes resembling *T. gambiense*; but without information about their infectivity to man little practical significance attaches to these organisms.

(2) *Recent observations on the behaviour of* T. gambiense *in antelope*

There is here but little positive evidence to be recorded—a confession the more regrettable because a stage has now been reached where tests on man himself can be safely and satisfactorily applied.

Three situtunga, two oribi, and one reedbuck, and a hybrid between bushbuck and situtunga, are the animals which have been exposed to *T. gambiense*. The experiments performed were as follows:

(*a*) SITUTUNGA (young male). Brought to the laboratory as an unweaned kid. No trypanosomes demonstrated by repeated examination of stained blood films, and by two subinoculations, each of 10 c.c. of blood, into clean monkeys. Exposed to laboratory-bred *G. palpalis* infected with *T. gambiense* (Strain XXVIII) in July 1928. Died with symptoms of enteritis on 17. viii. 29, *post-mortem* examination revealing no signs of trypanosomiasis.

The animal was fed upon by clean laboratory-bred *G. palpalis* as follows:

Test	Date	Total flies dissected	Total infected	%
1	12. viii–2. ix. 28	402	1	0·2
2	10–28. x. 28	428	1	0·2
3	26. i–11. ii. 29	238	0	0

No gland infections appeared in these flies (Duke, 1931).

(*b*) SITUTUNGA (female, young adult). Examined by the usual methods and no trypanosomes found. On 26. iv. 33, was bitten by four *G. palpalis*, each with heavy gland infections of *T. gambiense* (Strain LII). The same four flies had previously infected two sheep and a monkey, and in one of these sheep the trypanosome remained in a form transmissible by *G. palpalis* for at least 9 months. This situtunga never showed any signs of trypanosomiasis. Stained thick films were examined daily for 21 days after the exposure, and on 20. vii. 33 10 c.c. of the antelope's blood failed to infect a clean monkey. The animal lived for some 2 years after inoculation, and during that time gave birth to two hybrid kids by a bushbuck ram.

(*c*) SITUTUNGA (young male). Between May and July 1934 three injections, each of 10 c.c. of the antelope's blood, were made into clean monkeys, none of which became infected. On 2 and 3. iii. 35 this situtunga was bitten by four *G. palpalis*, all carrying a heavy infection with *T. gambiense* (Strain LXII). These

flies infected a human volunteer and a monkey after biting the antelope. Stained thick films of the antelope's blood were examined on numerous occasions from the tenth day after exposure to infection, but no trypanosomes were found.

On 19. iii, 5. iv, and 15. v. 35, 10 c.c. of this animal's blood failed to infect clean monkeys. Apparently this situtunga never became infected in spite of a very intense exposure to *T. gambiense*. It was eventually infected with *T. rhodesiense* by *G. palpalis*.

Thus of three situtunga used in these experiments, one developed a transitory infection, the trypanosome in the antelope failing to infect the glands of any of 1068 laboratory-bred *G. palpalis* which fed on it. The other two animals failed to become infected. Three different strains of *T. gambiense* were used, one for each animal. All these strains showed the characteristics of the mild type of East African *T. gambiense*, which produces a chronic disease in monkeys and guinea-pigs and, as far as is known, in man himself.

(*d*) ORIBI (female). Arrived at the laboratory as an unweaned kid from a tsetse-free area in the Eastern Province in February 1933. Preliminary examinations carried out with stained thick blood-films revealed no trypanosome infection.

On 21. iv. 33 was bitten by five and on 25. iv. 33 by a 6th *G. palpalis*, all of whose salivary glands were heavily infected with *T. gambiense* (Strain LI, isolated from a European some 2 months earlier). On 12. v. 35 trypanosomes were first seen in stained thick films of the animal's blood.

The following transmissibility tests with *G. palpalis* were applied to this oribi:

Test	Date	Total flies dissected	Total infected	% infected	Remarks
1	30. v–17. vi. 33	332	4	1·2	1 gland infection
2	26. ix–10. x. 33	262	1	0·3	No gland infection

On 26. ix. 33 and 27. ii. 35, 5 c.c. of the blood of this oribi failed to infect clean monkeys. The animal died on 28. ii. 35.

The latest evidence of infectivity is thus the second transmissibility test, *i.e.* Sept.–Oct. 1933. The animal was unwell for a day or two before it died. No necropsy was performed, but the day before death this antelope's blood proved non-infective to a monkey. It is therefore highly improbable that death was due to trypanosomiasis.

(*e*) ORIBI (female). Arrived in February 1933, as an unweaned kid. Usual preliminary examinations negative. On 8. v. 33 was bitten by three *G. palpalis* all of whose salivary glands were heavily infected with a strain of *T. gambiense* sent to the laboratory by Dr Van Hoof from the Belgian Congo. This strain had been found by Van Hoof to be strongly resistant to arsenic when first isolated from man. Trypanosomes appeared in the blood of the oribi on 13. v. 33. On 27. ix. 33, 5 c.c. of the animal's blood infected a clean monkey with *T. gambiense*. This oribi never showed any symptoms of illness and was not handled again until 11. iii. 35. On that date it was infected, by fly-bite, with *T. rhode-*

siense; it died of uncomplicated trypanosomiasis, after being obviously ill for 10 days, on 11. v. 35.

T. gambiense apparently caused this animal no inconvenience, the infection persisting for at least 4 months.

(*f*) REEDBUCK (male). Arrived as a kid at Entebbe in February 1933 from a tsetse-free area in the Eastern Province. This animal was originally set aside as a control in the antelope house at the Institute, and its history is given in a previous paper (Duke, 1934*a*). It became infected in August 1933 with *T. rhodesiense*, in all probability from three infected reedbuck companions, by the agency of *Stomoxys* in the antelope house. Inoculations of this reedbuck's blood into clean monkeys were made on 5. ix. 33 and 1. xii. 33 and on both occasions the monkey became infected with *T. rhodesiense*. An inoculation of 10 c.c. of the antelope's blood on 20. iii. 35 failed to infect a monkey. On 24. iii. 35 this reedbuck received subcutaneously 3 c.c. citrated blood of monkey 1362 (*T. gambiense*, Strain LXII)—living trypanosomes being present in the inoculum. On 15. iv. 35, 10. viii. 35, and 12. viii. 35, 10 c.c. of the antelope's blood were inoculated into clean monkeys, but no infection ensued.

In August 1935 the animal was exposed to flies infected with *T. rhodesiense* and 10 days later inoculation of 1 c.c. of its blood into a clean monkey produced infection with this trypanosome. The infection with *T. gambiense* appears to have died out between December 1933 and March 1935.

(*g*) HYBRID. Conceived at the laboratory and born on 5. vii. 34. Numerous preliminary blood-examinations revealed no trypanosomes. On 28. iii. 35 received under its skin the complete glands of four *G. palpalis* carrying *T. gambiense* (Strain LXII). All the flies showed heavy gland infections and were killed immediately before dissection. No trypanosomes were ever found in numerous stained thick films of the animal's blood, and inoculations into clean monkeys made on 16. iv. 35, 29. iv. 35, 17. v. 35, and 30. vii. 35 proved negative. The antelope therefore failed to become infected, although the inocula were extremely rich in living metacyclic trypanosomes.

(3) *Discussion*

Limited though they are, these observations on the behaviour of *T. gambiense* show that this trypanosome is but poorly adapted to life in the species of antelope chosen. The difficulty in securing an adequate number of gland-infected flies carrying *T. gambiense*, and the prior calls of other investigations, unfortunately prevented a more extensive examination.

It must be realised that *T. gambiense* in its ordinary East African form is much more difficult to work with than *T. rhodesiense*, at all events under the conditions prevailing at Entebbe. The trypanosome is less easily detected and is as a rule less readily transmissible by *G. palpalis*. The experiments described above are however fairly consistent in their results, and the conclusions drawn are, I think, correct for the particular strains used—strains which are typical of the organism responsible for Sleeping Sickness in Uganda.

From the foregoing observations it is evident that recent investigations in Uganda point to conclusions different from those acquired in 1912 at Mpumu. The difference must be due to the characters of the strains used. The nineteen strains of *T. gambiense* studied by the League of Nations Commission differed considerably from one another in their infectivity to mammals and transmissibility by tsetse, and had they been tested in game animals it is probable that differences would still have been apparent. A strain such as No. V of the Uganda series (origin, South Kavirondo, Lake Victoria) accommodated itself readily to sheep and might well have proved equally adaptable to antelope.

But in East and Central Africa, where *G. palpalis* is by far the most important vector of *T. gambiense*, ungulate game-animals are not as a rule common in inhabited *palpalis* country. From personal observation I believe that reptiles, hippopotamus, wild pig, and man and his domestic stock, form the main natural food supply of this tsetse. Certain species of antelope, elephant, and buffalo will also play a part in the fly's economy, but these species tend to avoid human settlement. Moreover, the little that is known of trypanosome infections of pig, hippo, and elephant indicates that they are unlikely to serve as reservoirs to *T. gambiense* (Duke, 1933).

On the whole, I think we must conclude with Wenyon—in his review of the situation presented in 1926—that *T. gambiense* in ordinary circumstances depends mainly on man and perhaps, but to a much less degree, on his domestic animals for survival in nature. It is most improbable that game animals are a danger in the spread of this trypanosome in nature.

The situation, referred to by Wenyon, on the uninhabited Sesse Islands of Lake Victoria is in several ways unique. Proof is no longer obtainable about my views (Duke, 1921) on the history of these lake-shore trypanosomes: at the time those views were forming, the crucial test on man had little to commend it. If *T. gambiense* ever does take up an exclusive existence in antelope, these lake-shore strains of "*mild T. brucei*" are the form one would expect it to assume. There is evidence, both general and experimental, that these strains are not at the present day infective to man (Duke, 1932).

My experience of typical *T. gambiense*, mild in its action in mammals, is that this trypanosome is prone to lose the power of developing in fly if it is kept for long in ruminants. The difference in this respect between *T. gambiense* and *T. rhodesiense* is noteworthy and has been recorded elsewhere (Duke, 1934). The contrast between the two trypanosomes will be seen when we examine *T. rhodesiense* later in this series of papers. In the meantime it must be conceded that the behaviour of *T. gambiense* recorded above certainly does not suggest that it can remain for long in ruminants in a form transmissible by tsetse.

It is probable also that domestic stock are a safeguard rather than a menace to their owners in *gambiense* sleeping-sickness areas. Over a huge extent of such territory in Africa the disease is, I believe, spread by a relatively exceedingly small number of infected tsetse. Domestic animals are therefore likely to

act rather as buffer between the trypanosome and man, both by satisfying the fly and by stifling (figuratively) the trypanosome.

There can be no reasonable doubt nowadays that man is the principal reservoir of *T. gambiense*, and that compared with him domestic and game animals are negligible. It is much to be regretted that the earlier researches carried out by Kleine and others (including myself) did not comprise an investigation of the infectivity to man of the strains examined. In view of recent discoveries about *T. rhodesiense*, information about *T. gambiense* in this respect would be of both academic and practical interest.

(4) *Conclusion*

The behaviour of *T. gambiense* in the antelope used in the experiments described in this paper suggests that—in its typical form in East and Central Africa—this trypanosome is unsuited to prolonged survival in situtunga, oribi, or reedbuck.

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STUDIES OF THE EFFECT ON *T. GAMBIENSE* AND *T. RHODESIENSE* OF PROLONGED MAINTENANCE IN MAMMALS OTHER THAN MAN, WITH SPECIAL REFERENCE TO THE POWER OF THESE TRYPANOSOMES TO INFECT MAN. II.

By H. LYNDHURST DUKE

The effect of prolonged maintenance away from man on the infectivity of *T. gambiense* for man

In this section are collected all the instances recorded at this laboratory of the testing of *T. gambiense* on man, after the trypanosome had been maintained for a time in laboratory animals.

(1) *Strain LXI.* Isolated from a Uganda native on 27. vii. 33. Two flies carrying this strain infected a volunteer on 7. xi. 33, the incubation period being 9 days.

(2) *Strain "Braun".* From Prof. Reichenow's Laboratory, Hamburg. Isolated from a patient from Fernando Po on 25. ii. 20 and maintained in laboratory rodents. Citrated blood of a guinea-pig infected with this strain was injected into a volunteer on 15. ii. 34 and trypanosomes appeared in his blood 6 days later. This strain was at the same time found to be non-transmissible by *G. palpalis.*

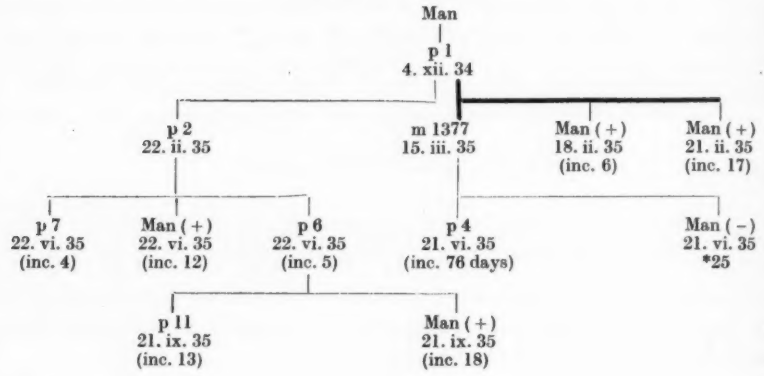
(3) *Strain LII.* From a native of the Katwe area, Western Uganda. Isolated in 2 monkeys from the patient on 8 and 9. iii. 33. After 4 passages through guinea-pigs it was tested on 2 volunteers, both of whom developed trypanosomes in their blood, the incubation periods being 9 and 8 days.

(4) *Strain "Br".* From Prof. J. G. Thomson's laboratory, London. Isolated from a European in Nigeria on 14. vi. 30. This strain showed some of the characters normally associated with *T. rhodesiense*, being in Prof. Thomson's opinion halfway between the two types. On 13. iv. 34, 2 volunteers and on 24. iv. 34 a third were inoculated with the citrated blood of a guinea-pig infected with this strain. They all became infected 10–13 days later.

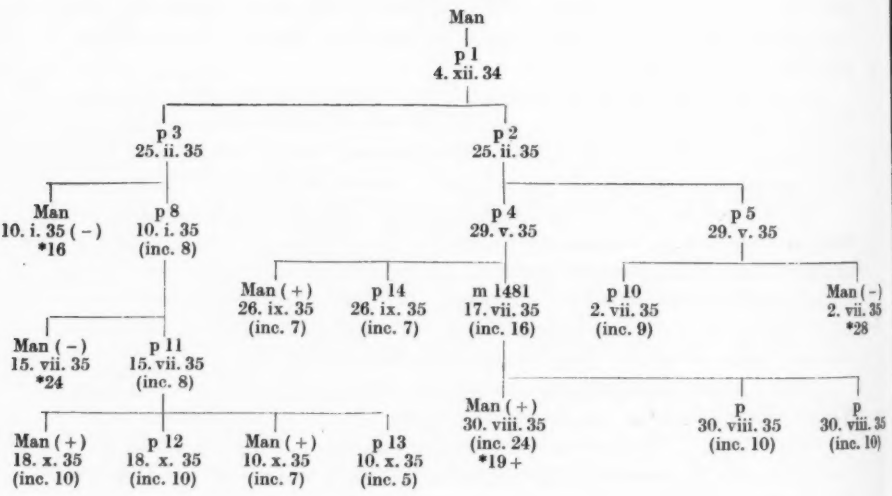
All the above experiments have already been described in full elsewhere (Duke, 1935).

Strain "Aga". Isolated on 4. xii. 34 from a native of the West Nile Province, Uganda, by inoculation of the patient's blood into a guinea-pig. A single fly carrying this strain infected 2 volunteers on 4 and 7. iii. 35 respectively. On 2. vii. 35, 210 days after its first infection, the blood of the original guinea-pig was inoculated into another volunteer and trypanosomes were found in his blood 9 days later.

Strain "Salim"



Strain "Kdy"



Strain "Salim". From a patient in the West Nile district of the Uganda Protectorate. The signs used in the following schedules are those described in earlier papers (Duke, 1935), the incubation period in days being added in certain instances. An asterisk followed by a number shows that an inoculation of the volunteer's blood was made on that day into a clean monkey; if positive, a + sign follows. As with most of the *T. gambiense* strains at this laboratory, trypanosomes were numerous in the guinea-pig after a few passages, but the animals lived for several months.

It will be noted that only one inoculation into man failed, but in the control guinea-pig more than 2 months elapsed before trypanosomes were detected. This negative result may therefore be disregarded.

Strain "Kdy". From a native of the West Nile area, Uganda. This schedule contains some remarkable records. Strain Kdy is the only strain of *T. gambiense* hitherto examined which has showed unequivocal signs of weakness in its pathogenicity to man. Its action in this respect is irregular, but the failures to infect man were definite and adequately controlled, and comprised 3 different men. The strain is apparently near the border-line, and its behaviour resembles certain strains of *T. rhodesiense* to be described in the next instalment.

CONCLUSIONS

Of 7 strains of *T. gambiense* tested in man after maintenance for various periods away from all contact with man, 6 retained apparently unimpaired their power of infecting him, although the number of volunteers employed with some of the strains was small.

The seventh strain failed to infect on 3 occasions and with 3 different volunteers, but infected 4 others.

THE INFECTIVITY OF "WILD" *T. BRUCEI* FOR MAN

The following is a brief summary of the tests carried out with *T. brucei* at the Entebbe Institute. Two new strains have been tested since the last publication on this subject.

(1) *T. brucei*. Strain LVI (*a*, *b*, and *c*); these 3 strains, recovered from wild *G. palpalis* on Damba Island, Lake Victoria, were tested on 4 volunteers (1 European and 3 natives). The men were exposed freely to laboratory-bred *G. palpalis* infective with these strains, and in no case did infection of man occur (Duke, 1935).

(2) *T. brucei*. Strain LIII; from the Kazinga channel in Western Uganda (*G. pallidipes*). A fly infective with this strain bit but failed to infect a volunteer.

(3) *T. brucei*. Strain LV; from the *G. morsitans* country in the vicinity of the Murchison Falls on the Victoria Nile, in the Northern Province of Uganda. Four gland-infected flies bit a volunteer but failed to infect him.

(4) *T. brucei.* Strain LVII; from the *G. pallidipes* country on the north-east shores of Lake Victoria. Flies infective with this strain failed to infect a volunteer.

(5) *T. brucei.* Two strains recovered from a *G. palpalis*-infested shore-line of Lake Victoria, near Entebbe. The strains were obtained in monkeys by exposing the animals to the bites of wild tsetse. The citrated blood of these monkeys was then inoculated into a volunteer, who did not become infected. Control animals inoculated at the same time became infected with *T. brucei.*

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THE PARASITES OF THE MUSKRAT (*ONDATRA ZIBETHICA* L.) IN THE BRITISH ISLES

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Introduction

Since 1914, when Mrázek urged that more attention should be paid to the parasites of the muskrat, a considerable number of helminths, many of them new, have been described from this host in North America. Little work has been done on muskrat ectoparasites. References in the literature to the parasites of the Central European muskrat are meagre.

The present work formed part of an investigation into the biology of the muskrat made possible by a grant from the Ministry of Agriculture and Fisheries to the Bureau of Animal Population, University Museum, Oxford. Most of the work was done at a temporary field station at Shrawardine, Shrewsbury, Shropshire, from August 1933 to September 1934. During this time muskrats were also obtained from Surrey, West Sussex, Perthshire, Stirlingshire and Tipperary through the kindly co-operation of officials of the muskrat campaigns concerned.

I am indebted to Dr H. A. Baylis, Zoology Department, British Museum (Natural History), for the identification of helminths and for advice; Miss A. Bishop, Molteno Institute, Cambridge, for examining smears for Protozoa; Mrs A. M. Hughes, Zoology Department, British Museum (Natural History), and Mr H. Britten, Manchester Museum, for the identification of lice, fleas, ticks and mites. Baylis (1935) and Bishop (1934) have already listed and where necessary described the helminths and Protozoa found in the present investigation.

Protozoa

It was only found possible to examine two muskrats for Protozoa immediately after death, as the great majority of the animals drown on capture and under field conditions were not usually obtained for several hours afterwards. In the caecum and colic loops of one of these *Trichomonas* sp., *Giardia* sp., *Chilomastrix* sp. and *Retortamonas* sp. were found (Bishop, 1934). Since the note describing these was published Miss Bishop has kindly examined smears from the intestine of a Perthshire muskrat obtained in November 1934. The duodenum, caecum and colic loops were negative for Protozoa. The small intestine showed a rather sparse fauna of *Trichomonas* sp. and one specimen

of *Retortamonas* sp. Hitherto retortamonads from mammals have only been found in the laboratory guinea-pig (Bishop, 1934) and *Cavia porcellus*, the Brazilian cavy (Cunha & Muniz, 1921), it therefore seems possible that this species has been introduced with the muskrat from America and has not been acquired in Britain.

Blood films proved negative, as did also a series of kidney smears stained and examined for spirochaetes.

Trematoda

One specimen of *Notocotylus quinqueserialis* Barker & Laughlin, 1911, was found in the caecum of a Shropshire muskrat in October 1933.

Cestoda

Some of the muskrats examined were infested with *Hymenolepis evaginata* Barker & Andrews, 1915, in the duodenum and small intestine. The number found per animal varied from one to eight with an average of five. In localities where it occurs the percentage of infection is high.

The muskrats found infested were trapped on marshes, ponds and backwaters of the rivers (where conditions approach those in ponds); in no cases were they obtained from rivers or large streams. Muskrats from some of the backwaters were free from this cestode, and animals which are known to have newly arrived at a pond or marsh were never found infected. This suggested that an intermediate host which does not live in running water is needed, but examination of Entomostraca (*Cyclops* spp. and ostracods) from an infected locality was negative, possibly owing to the low incidence of infection.

Table I. *Incidence and distribution of helminths of the muskrat* (Ondatra zibethica *L.*) *in the British Isles*, 1933–4

Locality	No. of muskrats examined	No. with *Notocotylus quinqueserialis*	% with *Notocotylus quinqueserialis*	No. with *Hymenolepis evaginata*	% with *Hymenolepis evaginata*	No. with *Taenia tenuicollis*, larval form	% with *Taenia tenuicollis*, larval form	No. with *Taenia taeniaeformis*, larval form	% with *Taenia taeniaeformis*, larval form	No. with *Capillaria hepatica*	% with *Capillaria hepatica*
Surrey and west Sussex	19	0	0	0	0	0	0	3	15·8	0	0
Shropshire	309	1	0·3	6	1·9	9	2·9	1	0·3	1	0·3
Perthshire and Stirlingshire	81	0	0	20	24·7	7	8·6	8	9·9	0	0
Tipperary	11	0	0	0	0	0	0	0	0	0	0

The high percentage of infection in Scotland (Table I) is accounted for by the fact that many of the muskrats there come from marshes, backwaters and ponds, while in Shropshire the great majority of the animals examined came

from the River Severn. *Hymenolepis evaginata* is apparently the only helminth introduced with the muskrat which has established itself in Great Britain to any extent.

Taenia tenuicollis Rud., 1819, larval form, and *T. taeniaeformis* (Batsch, 1786), larval form, occur in the liver, the former in Shropshire, Perthshire and Stirlingshire, the latter in these localities and also in west Sussex. A case of massive infection of over 100 larval *T. tenuicollis* in the liver was found, one case of mixed infection of both species of cysticerci occurred. Calcification had commenced in some cysts. The percentage of muskrats infested with cysticerci is much higher in Scotland than in Shropshire (Table I). As was previously pointed out, the habitats in these two areas are, generally speaking, different, and the varied incidences of infection may possibly be correlated with the habits of the final hosts.[1] Not only is the percentage infection lower in Shropshire than in Scotland, but also on the average fewer cysticerci were obtained in infested livers, and those found were of smaller size. The larva of *T. taeniaeformis* was reported from the liver of a Scottish muskrat by Cameron and Parnell (1933).

NEMATODA

Capillaria hepatica (Bancroft, 1894) was found in sections made by Dr G. M. Findlay of the liver of a Shropshire muskrat caught in March 1934. Irregular greyish patches showed in the liver surface. The liver was heavily infected with young worms which had not laid eggs. Around each nematode was an acute inflammatory reaction consisting of degenerating polymorphonuclear leucocytes. Damage to the liver was extensive, but the animal otherwise appeared to be in good condition. This parasite has only once before been recorded from the muskrat (Price, 1931*a*).

The small samples of animals originally imported from America, the possible loss of parasites during captivity, and the lessened chances of re-infestation (in many cases owing to absence of suitable intermediate hosts) may account for the small number of species and individuals of helminths found in the muskrat in Britain. Of the five species recorded, two (*Notocotylus quinqueserialis* and *Hymenolepis evaginata*) have hitherto only been recorded from North America, and so have probably been introduced with the muskrat. The other three species, *Capillaria hepatica*, *Taenia tenuicollis* (larval form) and *T. taeniaeformis* (larval form), have probably mostly been re-acquired from the local fauna. The helminth fauna recorded from the European muskrat is also sparse (Table II).

ECTOPARASITES

Ectoparasites were found on nearly all specimens of muskrats examined; sometimes they occurred in large numbers. Quite young muskrats were also infested. The species most commonly occurring are *Laelaps multispinosus*

[1] *Martes martes, Mustela erminea, M. nivalis, Putorius putorius, Canis familiaris* for *Taenia tenuicollis* and *Mustela erminea, Felis catus, F. sylvestris, Canis familiaris* for *Taenia taeniaeformis.*

Table II. *Distribution of muskrat parasites*

Endoparasites	North America	Europe	Britain	Hitherto recorded only from muskrat
Protozoa				
Trichomonas sp.	—	—	×	—
Giardia sp.	—	—	×	—
Chilomastix sp.	—	—	×	—
Retortamonas sp.	—	—	×	—
Eimeria stiedae Lindemann, 1865	×	—	—	—
E. Ondatra-zibethicae Martin, 1930	×	—	—	—
Eimeria sp.	—	×	—	—
Trematoda				
Pseudodiscus zibethicus (Barker & East, 1915) Fukui, 1929	×	—	—	×
Fibricola craterum (Barker & Noll, 1915) Dubois, 1932	×	—	—	×
Alaria mustelae Bosma, 1931	×	—	—	—
Echinochasmus schwartzi Price, 1931	×	—	—	—
Echinoparyphium contiguum Barker & Bastron, 1915	×	—	—	×
Echinostoma revolutum (Frölich, 1802)	×	—	—	—
E. callawayensis Barker & Noll, 1915	×	—	—	×
E. coalitum Barker & Beaver, 1915	×	—	—	×
E. armigerum Barker & Irvine, 1915	×	—	—	×
Urotrema shillingeri Price, 1931	×	—	—	×
Plagiorchis proximus Barker, 1915	×	—	—	—
Psilostomum ondatrae Price, 1931	×	—	—	—
Nudacotyle novicia Barker, 1916	×	—	—	×
Paramonostomum pseudalveatum Price, 1931	×	—	—	×
P. echinum Harrah, 1922	×	—	—	×
Catatropis filamentis Barker, 1915	×	—	—	×
Notocotylus urbanensis (Cort, 1914)	×	—	—	—
N. quinqueserialis Barker & Laughlin, 1911	×	—	×	—
Cladorchis [*Stichorchis*] *subtriquetrus* (Rudolphi, 1814)	×	×	—	—
Monostomum affine Leidy, 1858	×	—	—	×
Paragonimus sp.	×	—	—	—
Fasciola hepatica Linnaeus, 1758	—	×	—	—
Cestoda				
Anomotaenia telescopica Barker & Andrews, 1915	×	—	—	×
Hymenolepis evaginata Barker & Andrews, 1915	×	—	×	×
Urocystidium gemmiparum Beddard, 1912	—	—	—	×
Taenia taeniaeformis (Batsch, 1786), larval form	×	×	×	—
T. tenuicollis Rudolphi, 1819, larval form	×	—	×	—
T. pisiformis (Bloch, 1780), larval form	—	×	—	—
Nematoda				
Trichostrongylus fiberius Barker & Noyes, 1915	×	—	—	×
Capillaria ransomia Barker & Noyes, 1915	×	×	—	×
C. hepatica (Bancroft, 1894)	×	—	×	—
Trichuris opaca Barker & Noyes, 1915	×	×	—	×
Filaria sp.	×	—	—	—
Physaloptera sp.	×	—	—	—
Rodentocaulus ondatrae Schulz, Orlow & Kutass, 1933	—	×	—	×
Longistriata dalrymplei Dikmans, 1935	×	—	—	×
Ectoparasites				
Gamasidae				
Laelaps multispinosus Banks, 1909	×	—	×	×
Ixodidae				
Ixodes banksi Bishopp, 1911	×	—	—	×
Trombidiidae				
Myobia n.sp.	—	—	×	×
Sarcoptidae				
Acarus siro var. *Canis* Linnaeus, 1758	—	×	—	—
Listrophorus sp.	—	×	—	—
L. validus Banks, 1909	×	—	×	×
Tyroglyphidae				
Tyroglyphus sp.	—	—	×	—
Dermacarus n.sp.	×	—	×	×
Anoplura				
Polyplax spiniger Burm.	—	×	×	—
P. spinulosus Burm.	—	×	—	—

Banks, 1909 = *Tetragonyssus spiniger* Ewing & Stover, 1915, *Listrophorus validus* Banks, 1909 and *Dermacarus* sp., homopial form, which Mrs Hughes of the British Museum considers to be new. Their incidence and distribution is given in Table III. During handling in the field before examination many ectoparasites would be lost, and but for this the incidence would doubtless be higher. All three species were present in a tube of material from an Ontario muskrat kindly sent me by Mr D. Chitty, Department of Biology, Toronto University. *Dermacarus* n.sp. has not previously been recorded from the muskrat. The adult form of *Dermacarus* n.sp. will probably be found in muskrat houses and nests; no opportunity for collecting such material presented itself.

As well as the above species one specimen of an adult tyroglyphid was obtained in Shropshire and one specimen each of *Polyplax* sp. and *Myobia* sp. from Scotland. Mr Britten of Manchester Museum considers this *Myobia* to be new. The first two of these are probably stragglers, the tyroglyphid may be the

Table III. *The incidence and distribution of the three most commonly occurring species of muskrat ectoparasites in Great Britain*

Place	No. of muskrats examined	No. with *Laelaps multispinosus*	No. with *Listrophorus validus*	No. with *Dermacarus* n.sp.	No. with 3 species	No. with 2 species	No. with 1 species
Shropshire	44	34	39	33	22	18	4
Surrey (Farnham)	1	1	1	0	0	1	0
West Sussex (Pulborough)	2	2	1	0	0	1	1
Scotland	8	4	6	1	0	3	5
Ireland	6	1	6	0	0	1	5

adult of *Dermacarus* n.sp. and the *Polyplax* is possibly an immature *P. spiniger* Burm. which occurs on *Arvicola amphibius amphibius* L., the water vole. Evidently then the American ectoparasite fauna has been introduced into Britain virtually in entirety[1] (Table II). Additions from the local fauna have been negligible, possibly owing to the aquatic habits of the muskrat and its neighbours the water vole and, to a less extent, the brown rat. In the case of terrestrial mammals, on the other hand, a greater interchange of ectoparasites seems to occur among those occupying the same habitats; thus Elton *et al.* (1931) found that several species of fleas and mites are common to *Apodemus*, *Microtus* and *Evotomys*. No muskrat ectoparasites were found on water voles and brown rats from muskrat-infested localities.

During the biological work on muskrats from 1933 to 1935 water voles (*Arvicola amphibius amphibius* L.) and brown rats (*Rattus norvegicus* Erx.) were examined for parasites to see what light they might throw on the parasitology of the British muskrat. Water voles were examined to a greater extent and more thoroughly than brown rats because of their closer biological association

[1] *Ixodes banksi* Bishopp, 1911, has been recorded on only one occasion from America.

with the muskrat. In brown rats the liver was the only organ examined for endoparasites.

It is hoped to deal with the endoparasites listed below in greater detail later.

Arvicola amphibius amphibius L.

Protozoa	Endoparasites	
Trichomonas sp.	Rectum	Found in one out of four specimens from Pentre (Shropshire)—the duodenums were negative. The *Chilomastix* is larger than the very small type found in the muskrat
Chilomastix sp.	Rectum	
Trematoda		
Plagiorchis sp.	Small intestine	Millhill, Braco, Perthshire
Cestoda		
(?) *Paranoplocephala blanchardi* (Moniez)	Duodenum	Millhill, Braco, Perthshire
Taenia tenuicollis (Rudolphi, 1819)	Larval form, liver	From a Shropshire specimen. Larval cestodes are not common in the water vole.
Nematoda		
Syphacia(?) *obvelata* (Rudolphi, 1802)	Caecum	Very common in water voles from all habitats in Shropshire and Perthshire. The males were seldom found
(?) *Gongylonema* sp.	Liver	From a Perthshire specimen, eggs alone were found, no trace of the parent worm
	Ectoparasites	
Laelaps agilis Koch		Common parasites in west Sussex, Shropshire and Perthshire. *Dermacarus* sp. from the water vole is distinct from *Dermacarus* sp. of the muskrat
Listrophorus leuckarti Pgst.		
Dermacarus sp.		
Eulaelaps stabularis Koch		Once recorded from Pulborough, west Sussex
Polyplax spiniger Burm.		Commoner in Perthshire than in Shropshire, also occurs in west Sussex
Ctenopthalmus agyrtes celticus (Jordan & Roths., 1922)		Shropshire
Ceratophyllus walkeri Roths.		Shropshire

Rattus norvegicus (Erxleben)

	Endoparasites	
Taenia taeniaeformis (Batsch, 1786)	Larval form, liver	Common in Shropshire and Perthshire. In the latter county 6·9 per cent. of 58 rats examined were infested
	Ectoparasites	
Eulaelaps stabularis Koch		A common parasite of the brown rat in Shropshire
Ixodes tenuirostris Neumann	Larvae	Found on two occasions in Shropshire
Notoedres muris Megn.		Scabs on ears in Shropshire
Ceratophyllus fasciatus Bosc.		Shropshire
Polyplax spinulosus Burm.		Shropshire

Summary

Of the five species of helminths of the British muskrat (*Ondatra zibethica* L.), two (*Notocotylus quinqueserialis* and *Hymenolepis evaginata*) have been introduced with the animal. The three other species of helminths (*Capillaria hepatica*, *Taenia taeniaeformis* larva and *T. tenuicollis* larva) have probably

been largely re-acquired from the local fauna. All of the three common species of ectoparasites [*Laelaps multispinosus* (*Tetragonyssus spiniger*), *Listrophorus validus* and *Dermacarus* n.sp.] occur in North America. Three other species (*Tyroglyphus* sp., *Myobia* n.sp., *Polyplax* sp.) have occurred as single individuals, the last species having probably been acquired from the water vole. Introduction into Britain has greatly depleted the original helminth fauna as regards number of species and individuals; but not so with the ectoparasites. Lists of the parasites hitherto recorded from the muskrat are given and those found in brown rats (*Rattus norvegicus* Erx.) and water voles (*Arvicola amphibius amphibius* L.).

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FURTHER EXPERIMENTS UPON THE LONGEVITY OF *XENOPSYLLA CHEOPIS* ROTHS. (SIPHONAPTERA)

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(With 3 Figures in the Text)

The experiments herein recorded are a continuation of previous work (Leeson, 1932). As the former experiments and those to be described are so closely connected they will be discussed together. In the original investigation the fleas were all unfed and under 24 hours old at the commencement of the experiments, and the main conclusions reached were that (1) duration of life of unfed fleas was not influenced by sex, (2) at any temperature they live longer at high humidities than at low, and (3) there was no direct relation between survival of unfed fleas and saturation deficiency at any temperature. Because of these conclusions which did not coincide with those of other workers, notably Bacot & Martin (1924) and Hirst (1926), it was decided to experiment with *Xenopsylla cheopis* which had been fed once or repeatedly. The objects were to discover if there were any material differences between the longevity of unfed and fed fleas; if feeding had any effect upon survival of the sexes or upon the relationship between survival and saturation deficiency of the atmosphere.

The methods employed were essentially the same as those described in the former paper (Leeson, 1932), except that fleas which had fed once, or repeatedly, were used.

One set of experiments was performed with fleas which had fed once only and which were then starved under controlled conditions. The fleas were taken upon emergence from the cocoons and put aside in some debris, oats, etc., until the second day after collection. This was done because it was found that adults would not feed on the day of emergence, and that the next day only a small proportion would feed. But on the second day after emergence all the fleas would feed and immediately after they had done so they were put away into controlled atmospheres as in the previous experiments, each flea, by itself, in a small glass tube capped with gauze. The feeding was done on the shaved belly of a young rat.

In the second series of experiments, in which fleas were fed for 7 days, the stock jars were cleared of adults daily. These adults (unfed and under 24 hours old) were then put into a jar with a flea-free mouse; this was done daily with a fresh jar and mouse. The number of fleas put with each mouse was recorded (as nearly 100 as was possible), and each batch was left for 7 days. On the

seventh day all fleas among the debris were recovered and put into their experimental vessels in the same way as the others. Fleas actually on the mouse were removed by slightly anaesthetising the animal with chloroform. These fleas do not enter into the experiments, as they were killed.

Before proceeding to discuss the results of the experiments it is of interest to analyse the data regarding the numbers of fleas recovered after being kept with the mice for 7 days. The total number of fleas put with 19 mice was 1814. It was impossible for the fleas to escape, but only 1200, approximately 66 per cent., were recovered. The mice kill a certain number and some fleas die in the first few days of adult life, but the deaths in individual batches vary greatly. For example, one batch consisting of 84 fleas was reduced to 10 (approximately 12 per cent.) at the end of the week. At the other extreme is a batch of 60 fleas, 53 of which (approximately 88 per cent.), were recovered.

With regard to the situation of the fleas when recaptured 695 (approximately 38 per cent. of the total) were collected from the debris and 505 (28 per cent. of the total) from the mice. As percentages of recovered fleas the figures would be 57 and 42 per cent. respectively; thus the *total* number of fleas recovered from the debris is greater than that of the fleas off the mice. But on examining figures for the separate batches it was found that in 6 cases larger numbers were taken from the mice. Percentages of all recovered fleas found on mice varied from 17 to 89 per cent., and for those from the debris from 10 to 82 per cent.

Concerning sex, the figures cannot be related to the total fleas used or recovered because the number of fleas of each sex originally introduced is unknown. It was noted, however, that in two batches all the females recovered were from among the debris, while all the fleas on the mouse were males. Altogether 717 males were recovered, of these 396 (54 per cent.) were among the debris; of 483 females recaptured, 299 (62 per cent.) were among the debris. Generally speaking, both sexes were found in larger numbers among the debris than on the animal, but again some individual batches produced the opposite result.

Results of experiments and discussion

Temperature and humidity

Hopkins (1935), working with unfed, newly emerged *Xenopsylla cheopis* in Kenya at 20° C. and 100 per cent. relative humidity, obtained a mean survival of over 21 days. The nearest comparable conditions (18° C. and 90 per cent. relative humidity), studied by the present writer, produced a mean survival of only 14·6 days; experiments which resulted in a mean survival of 21 days were conducted at 12° C. and 90 per cent. relative humidity.

The apparent discrepancy may perhaps be accounted for by differences in technique, and needs investigation.

Survival times for the three groups of experiments to be discussed are included in Table I, the comparable figures for the unfed fleas being abstracted

Table I. *Summary of survival times (in days) of* Xenopsylla cheopis *Roths.*

	°C.	R.H. %	mm. Hg saturation deficiency	Both sexes: No. of fleas	Both sexes: Max. survivals	Both sexes: Mean survivals	Both sexes: *Standard deviations*	Males: No. of males	Males: Max. survivals	Males: Mean survivals	Males: *Standard deviations*	*P*	Females: No. of females	Females: Max. survivals	Females: Mean survivals	Females: *Standard deviations*
Unfed	23	30	14·7	154	13·5 ♂	4·8	*2·3*	87	13·5	5·1	*2·6*	—	67	9·5	4·4	*2·1*
		60	8·4	113	14·5 ♂	6·2	*2·6*	60	14·5	6·2	*2·8*	—	53	12·5	6·2	*2·3*
		75	5·3	98	13·5*	6·5	*2·7*	50	13·5	6·5	*2·6*	—	48	13·5	6·5	*2·8*
		90	2·1	140	21·5 ♂	7·3	*4·4*	70	21·5	6·3	*3·9*	*0·01–0·02*	70	20·5	8·3	*4·6*
	30	35	20·6	60	6·5 ♂	3·7	*1·4*	43	6·5	3·8	*1·5*	—	17	4·5	3·5	*1·3*
		50	15·9	107	8·5*	4·2	*1·5*	59	8·5	4·5	*1·6*	—	48	8·5	3·8	*1·5*
		75	7·9	68	10·5 ♂	4·4	*1·8*	34	10·5	4·7	*2·2*	—	34	6·5	3·8	*2·2*
		85	4·8	75	13·5 ♂	4·7	*2·4*	45	13·5	4·9	*2·9*	—	30	7·5	4·2	*1·4*
Fed once	21	25	13·9	67	12·5 ♀	8·1	*1·6*	28	11·5	8·1	*1·5*	*0·50–0·70*	39	12·5	8·1	*1·6*
		50	9·3	58	12·5*	9·1	*2·1*	36	12·5	9·1	*1·5*	*0·70–0·80*	22	12·5	9·1	*2·4*
		80	3·7	43	19·5 ♀	9·7	*3·2*	26	15·5	9·1	*2·6*	*0·50*	17	19·5	10·6	*3·8*
		90	1·9	130	20·5 ♂	10·3	*3·0*	86	20·5	10·4	*3·0*	*0·50–0·70*	44	18·5	10·0	*2·9*
	30	20	25·4	97	10·5 ♂	5·7	*1·1*	58	10·5	5·8	*1·2*	*0·70–0·80*	39	7·5	5·6	*0·8*
		50	15·9	65	11·5 ♀	6·6	*1·1*	37	8·5	6·6	*1·0*	—	28	11·5	6·7	*1·3*
		70	9·5	64	12·5 ♀	7·1	*1·8*	32	11·5	7·0	*1·6*	—	32	12·5	7·2	*2·0*
		90	3·2	102	14·5 ♂	7·7	*2·4*	56	14·5	8·1	*2·7*	*0·10–0·20*	46	11·5	7·2	*1·9*
Fed repeatedly	25	25	17·8	90	11·5 ♀	9·2	*0·9*	51	10·5	8·8	*0·8*	*<0·01*	39	11·5	9·7	*0·8*
		50	11·9	93	13·5 ♀	9·5	*1·1*	51	10·5	9·0	*0·8*	*<0·01*	42	13·5	10·1	*1·2*
		80	4·7	56	13·5*	10·7	*1·6*	35	13·5	10·3	*1·3*	*<0·01*	21	13·5	11·8	*1·2*
		90	2·4	298	17·5 ♀	11·2	*2·0*	188	16·5	10·9	*1·9*	*0·01–0·02*	110	17·5	11·7	*2·0*
	30	25	23·8	39	9·5 ♀	8·6	*0·6*	18	8·5	8·3	*0·4*	—	21	9·5	8·8	*0·7*
		50	15·9	64	10·5 ♀	8·6	*0·8*	35	9·5	8·2	*0·6*	—	29	10·5	9·2	*0·6*
		70	9·5	30	10·5 ♀	9·4	*0·8*	11	9·5	8·8	*0·6*	—	19	10·5	9·8	*0·6*
		90	3·2	120	16·5 ♀	10·4	*1·7*	42	12·5	9·1	*0·9*	*<0·01*	78	16·5	11·1	*1·5*

* indicates that individuals of both sexes survived equally.

Standard deviations indicate the degree of scatter around the mean survival times.

The figures under the column heading "*P*" are obtained from Fisher's table after applying the χ^2 test for significance as between survival times of the sexes. If the value of "*P*" is below 0·05 it may be taken that the difference between the sexes is significant, by conventional standards. If the value of "*P*" is above 0·05 the difference may be real, but might easily be due to insufficient sample.

from the previous paper (Leeson, 1932). It is seen once again that within the range of the experiments and for those fleas in the same state of nutrition the lower temperatures are more favourable to longevity than are the higher; also that at any one temperature fleas live longer at higher humidities. This latter observation is true whether the fleas are fed or unfed. The Figs. 1 and 2 illustrate this very effectively and further show that the proportionate increase as the higher humidities are approached is practically identical whatever the nutritional state of the insects, as the lines connecting survivals at the same temperature are straight and practically parallel.

Saturation deficiency

It has already been shown by arithmetical and graphical methods that there is no direct proportion between length of life of unfed fleas and saturation deficiency. If the same methods are applied to the present experiments on fed fleas there is found to be still no direct proportion. Table II shows the "expected" survivals calculated by dividing the product of the mean survival and the saturation deficiency at 50 per cent. relative humidity by the other saturation deficiencies. The figures show clearly that no relationship exists, neither does it exist for fleas fed repeatedly or kept at any other temperature. There is then no agreement with Bacot and Martin's conclusion that survival is directly proportional to saturation deficiency. It must be borne in mind that the three sets of figures are not comparable, because of the difference in age of the fleas at the commencement of the experiment; they must be studied independently.

Feeding

As the experiments could not be exactly repeated fed fleas were exposed at 21 and 25° C., while the unfed were exposed at 23° C. The fed fleas lived much longer at both temperatures, but it is better to examine the figures obtained at 30° C. where precise comparison is possible. It is then seen that those given a blood meal to begin with lived longer than those unfed. In fact, fed fleas kept at 30° C. lived much longer than unfed fleas kept at 23° C., 7° lower. It may be objected that the survival times were influenced by selection, because only those fleas were used which had survived 2 and 7 days respectively. Nevertheless, it would appear from the figures and facts given here that if, out of a population of fleas, some manage to obtain a meal before a period of starvation they will live longer than those which do not. Another point which emerges from this investigation is that while an initial single meal of blood results in an extension of life, a continuous period in association with a host which allows the fleas to obtain a meal when and as often as they like before a period of starvation, permits of a still further extended existence.

Experiments recorded by several authors employing wild-caught fleas off rats cannot be compared in this section as the ages of the insects were unknown. They will be referred to below in the observations on sex.

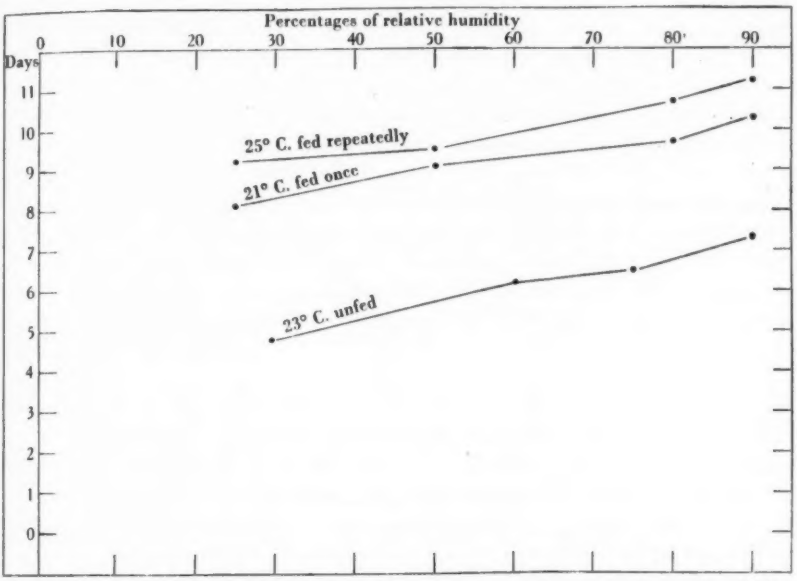

Fig. 1. Mean survivals in days of fed and unfed fleas (*Xenopsylla cheopis*) at different humidities and temperatures; survivals at the same temperatures are connected by lines.

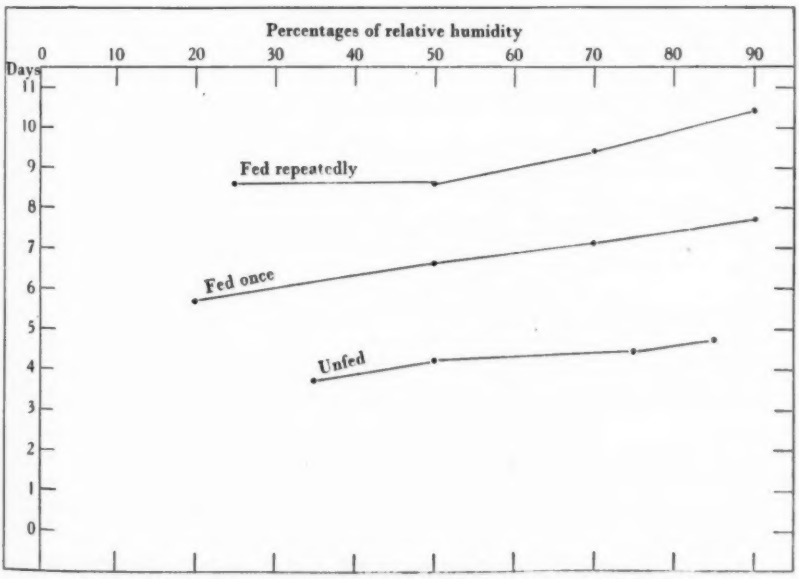

Fig. 2. Mean survivals in days of fed and unfed fleas (*Xenopsylla cheopis*) at 30° C. and different humidities.

Sex

It has already been seen that so far as unfed fleas are concerned survival is not influenced by sex. This has recently been confirmed by Hopkins (1935). Inspection of the mean figures for males and females separately in the experiment upon fleas fed once leads one to a similar conclusion. The application

Table II. *Mean and "expected" survivals in days of fed* Xenopsylla cheopis *at* 21° C. *at different humidities*

Fleas fed once

° C.	R.H. %	Saturation deficiency mm.	Mean survivals days	"Expected" duration of life days
21	25	13·9	8·1	6·0
	50	*9·3*	*9·1*	*9·1*
	80	3·7	9·7	22·8
	90	1·9	10·3	44·5

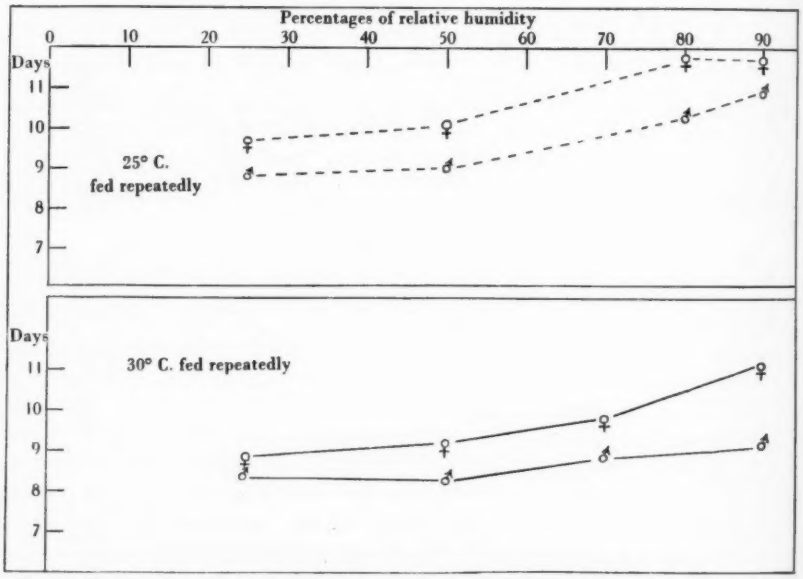

Fig. 3. Mean survivals in days of fleas (*Xenopsylla cheopis*) fed repeatedly before starvation showing difference in mean survivals of the sexes.

to the results of the χ^2 test for significance confirms this, and there is no suggestion that any significant difference exists between the survival times of the sexes.

With fleas fed for 7 days before starvation the case is obviously different. In each instance females lived longer than males (Fig. 3). The statistical

significance of the differences between the sexes has been tested (by χ^2), and they appear so great that they must all be regarded as significant.

Bacot & Martin did not distinguish between sexes, but Bacot (1914), Hirst (1926) and Webster (1930) found that females lived longer than males when fed. Likewise Hopkins, when using wild-caught fleas of unknown age, clearly showed that under identical atmospheric conditions the mean survival of females is longer than that of the males. According to the present work it would appear that one feed before starvation, while sufficient to extend the period of survival, does not influence one sex to live longer than the other. On the other hand, when association with a host for some days precedes a time of food shortage, females definitely acquire a greater resistance to starvation than do the males.

SUMMARY AND CONCLUSIONS

1. Experiments upon *Xenopsylla cheopis* Roths. are described which were carried out to discover if feeding influenced duration of life or had any effect upon the survival of the sexes or upon the relationship between duration of life and saturation deficiency of the atmosphere.

2. Fleas fed once before starvation lived longer than unfed fleas. There was no difference between survival of the sexes and no direct proportion between survival times and saturation deficiency.

3. Fleas kept with the host for 7 days before starvation lived still longer. Females lived considerably longer than males, but there was still no direct proportion between survival times and saturation deficiency.

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SOME RARE AND NEW PARASITIC COPEPODA, ETC., FROM BRIGHTON AND ELSEWHERE

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.)

(With 2 Figures in the Text)

The following animals are from a collection made for and kindly presented to me by Miss Miriam Rothschild during her visit to Brighton in July and August 1935.

PYCNOGONIDA

Pycnogonum littorale (Stroem)

Host. *Gadus merlangus.*
Material. 1 specimen.
Location. Gill.
Locality. Brighton.
Remarks. C. L. Walton records that young specimens are parasitic at Plymouth on an anemone, but this is the first observation of an adult on the gills of a fish.

DICHELESTHIIDAE

Nemesis robusta van Beneden

Host. *Alopias vulpinus* (Bonnaterre) = *A. vulpes* (Gmelin).
Material. 21♀♀.
Location. Gills.
Locality. Brighton.
Remarks. New Host. Second record for English waters.

LERNAEIDAE

Lernaeocera lusci (Bassett-Smith)

Host. *Brosme brosme.*
Material. 1♀.
Location. Gill.
Locality. Morocco.
Remarks. New host. New locality.

CALIGIDAE

Caligus rabidus n.sp.

Host. *Alopias vulpinus.*
Material. 1♀.
Location. Cloaca.
Locality. Brighton.

Diagnosis (Fig. 1). *Cephalothorax* short, as wide as long, anterior margin scarcely rounded, frontal platform large, posterior sinuses shallow. *Genital segment* pear-shaped, narrow in front, much longer than wide. *Abdomen* of one

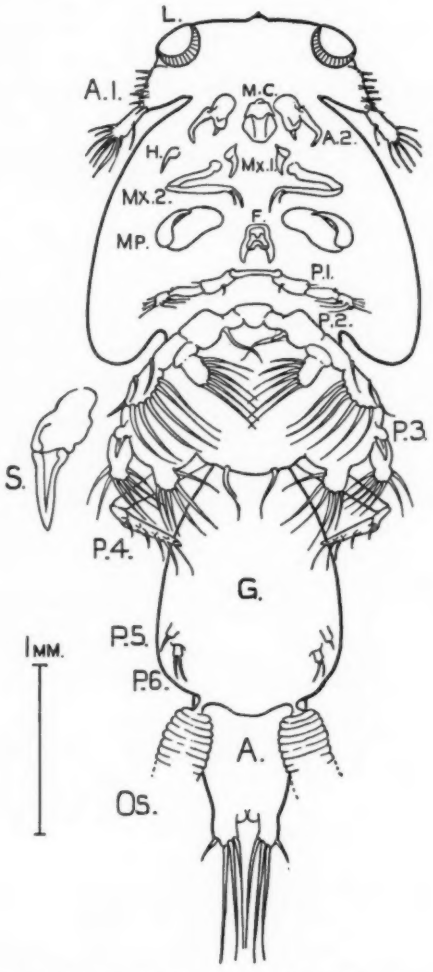

Fig. 1. *Caligus rabidus* n.sp. ♀ in ventral aspect. *L.* = lunules; *M.C.* = mouth cone; *P.* 1–6 = pereiopods; *S.* = spike on third pereiopod; *G.* = genital segment; *A.* = abdomen; *Os.* = ovisacs. Other lettering as in Fig. 2.

segment longer than wide, abdominal appendages well marked with three long and one short outer setae. The boundary between the genital segment and the abdomen is convex posteriorly.

Appendages (Fig. 2). *First antenna* large, two-articled, terminal article nearly as long as basal; both articles very setose.

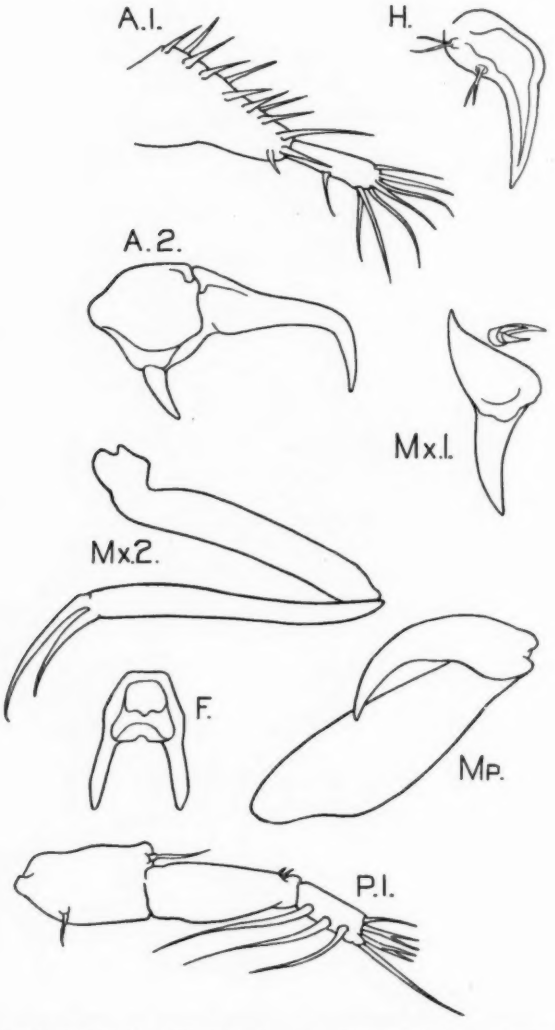

Fig. 2. *Caligus rabidus* n.sp. ♀. Appendages. *A.* 1=antennule; *A.* 2=antenna; *Mx.* 1=first maxilla; *Mx.* 2=second maxilla; *H.*=maxillary hook; *F.*=furca; *Mp.*=maxillipede; *P.* 1=first pereiopod.

Second antenna as is usual in this genus, a long curved claw with a supplementary hook on the posterior margin of the basal article.

First maxilla entirely posterior to mouth cone, an unusual feature in this genus, short, slightly curved, a spinous process not far from the base.

Maxillary hook (*H.*) of the same size as the first maxilla and parallel with it, bearing on its base two setae as in *C. dubius* Scott, but in this case one is completely and the other partially bifurcated.

Second maxilla slender, two-articled, distal article equal in length to the basal; the two curved claws at the apex unequal.

The details of the *furca* (Fig. 2, *F.*) are unusual and ornate and difficult to express in words.

Maxillipede stout, two-articled, the terminal claw only about half the length of the basal article which exhibits no socket or process for the claw to fit down upon.

The *first pereiopod* is three-articled (Fig. 2, *P.* 1); the proximal article bears two setae, the middle is the longest, and the terminal is furnished posteriorly with three long plumose setae and apically with four setae, the posterior of which is very long and the two central bifurcated, a condition only as yet recorded in one British species *C. zei* Norman and T. Scott (*vide* Oakley, 1926) and in three American species which are very unlike the present one.

The spike (Fig. 1, *S.*) on the expodite of the *third pereiopod* is large.

The terminal article of the *fourth pereiopod* bears five long setae posteriorly.

The *fifth and sixth pereiopods* are plainly visible; the sixth is the larger with two setae, the fifth having but one.

The eyes are red.

The following characters may be regarded as specific: The shape and size of the genital segment and abdomen and the ornate appearance of the furca; the presence of bifurcated setae on the first pereiopod, and of two bifurcated setae on the maxillary hook.

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OBSERVATIONS ON THE SPOROZOA INHABITING THE GUT OF THE POLYCHAETE WORM *POLYDORA FLAVA* CLAPARÈDE[1]

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From the Department of Zoology, King's College, University of London

(With 22 Figures in the Text)

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I. Introduction

The occurrence of Coccidia in annelid worms is rare enough, but the coccidian[2] from the intestine of *Polydora flava* is even more remarkable in being an intranuclear parasite; and it is to this parasite that special attention is directed in the following account.

The Sporozoa to be found in the gut of the same worm comprise two other species: one a new species of *Selenidium*, which I propose to name *Selenidium axiferens*, and a dicystid gregarine *Polyrhabdina polydorae* Caul. & Mesn. (?). Some account is given of the morphology and life history of *Selenidium axiferens*, and a few notes on the dicystid are appended.

II. Material and methods

The material came from Rum Bay, Plymouth, and the following observations were made in London for the most part; the few weeks I spent at Plymouth were occupied in making smears and studying oocysts, which were kept under aeration in moist chambers.

Before fixation, the worms were narcotised by adding crystals of magnesium sulphate to the sea water containing them, and then enclosed between

[1] Thesis approved for the Degree of Master of Science in the University of London.

[2] Dr H. N. Ray, of the University College of Science and Technology, Calcutta, rediscovered this parasite, and I began my work on some material that he had left in the Zoology Department of King's College, London.

strips of glass. Without such precautions, it is impossible to prevent the worms coiling up. Serial sections of 80 worms were cut and examined, sections 6–8 μ thick being found most useful.

Alcoholic Bouin's fluid was the fixative mainly employed, though some of my material was treated with Brasil's modification of Bouin and Duboscq's. The stains used were Heidenhain's iron-haematoxylin with chromotrope 2R as counterstain, Dobell's modification of Mann's methyl blue-eosin, Delafield's haematoxylin with eosin or chromotrope 2R, and safranin with light green. Gentian violet and iodine were used as suggested by LaCour (1931), but I sometimes varied this method by strongly differentiating with clove oil. Feulgen's nucleal reagent has also been used, but only with partial success: for although the nuclei of the cells of the host stained admirably, it was extremely difficult to obtain satisfactory pictures of those of the parasites.

III. Description of the Sporozoa

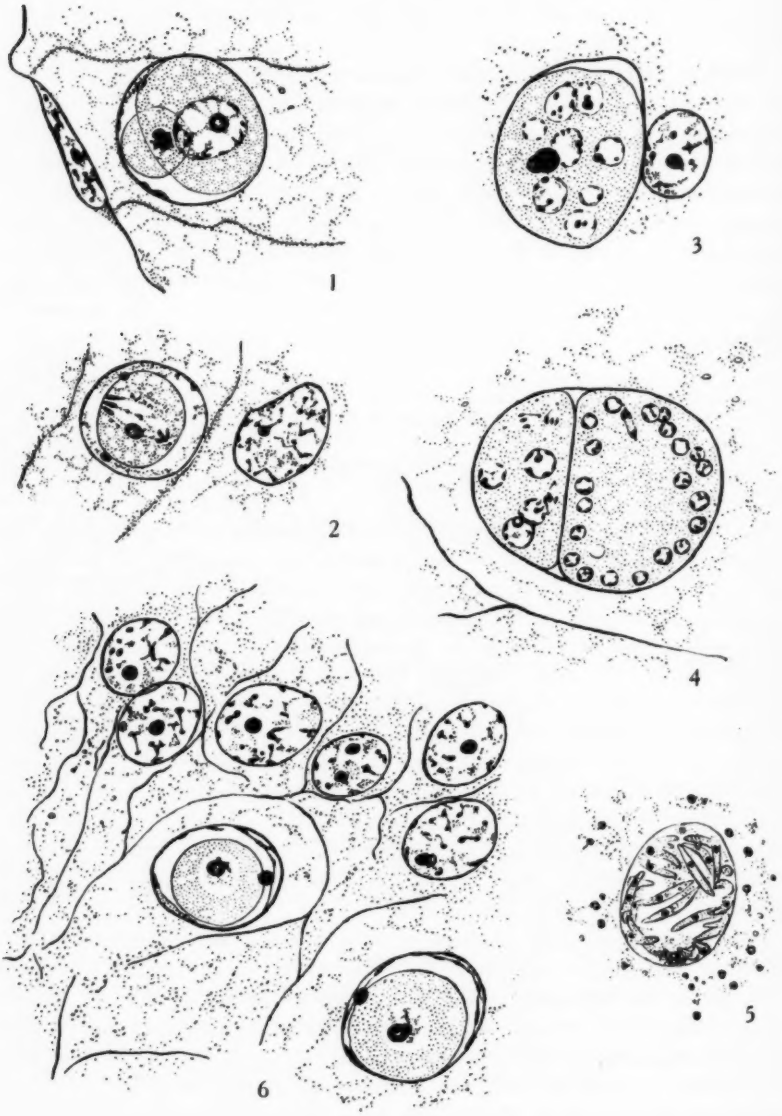
(i) *The coccidian*

(*a*) *Occurrence*

In 1897, Caullery & Mesnil recorded a coccidian from *Polydora flava*, but they evidently gave it little more than a superficial examination, for, in stating the locus of infection, they inserted a mark of interrogation after the word "intestine". Not only is it confined to the gut, but it is strictly intranuclear in the schizogonic as well as in the sporogonic phase of the life cycle. Intranuclear coccidia have been described before, but, so far as I am aware, there are only three records of exclusive intranuclear parasitism—*Cyclospora caryolytica* Schaudinn (1902), *Isospora mesnili* Sergent (1902), and *Eimeria ranarum* Labbé, Laveran & Mesnil (1902)—all parasites of vertebrates. In *Dobellia binucleata* Ikeda (1914), where the sporozoites are said to be sexually differentiated, one type of sporozoite is intranuclear in the early stages of its growth. Other coccidia, such as *Eimeria salamandrae* Steinhaus,[1] are facultative intranuclear parasites: that is to say, they may occur as often in the cytoplasm of the infected cells as in the nuclei.

The infection is chiefly confined to the middle and posterior regions of the intestine. The parasite appears to have no notable pathological effect on its host, despite its destruction of the nuclei. The contents of an infected nucleus are soon dissolved, and in early stages of growth the coccidian lies within a vacuole bounded by the nuclear membrane (Fig. 6); the peripheral layer of nucleoplasm may persist for a short time, and the remains of the chromatin may often be seen, up to a late stage of development, as a small dense mass (Fig. 11). Sometimes 3 or 4 parasites (or even more) may be found within

[1] It is interesting to recall that the intranuclear parasitism of *Eimeria* (*Karyophagus*) *salamandrae* enabled Simond (1896) to identify schizogony and sporogony as developmental stages of one and the same parasite: a striking confirmation of the view, first put forward in 1891 by Pfeiffer, that a coccidian passes through two different cycles of development in its life history.



Figs. 1–6.

All drawings were made with the aid of the camera lucida, and the magnification is ×2000 unless otherwise stated. All figures are of sections of material fixed in alcoholic or modified Bouin's fluid; and stained with iron haematoxylin, with chromotrope 2R as counterstain, unless the contrary is stated. The cilia of the epithelial cells of the host have been omitted.

the same nucleus; and, as they grow, they become flattened on their apposed surfaces. Apart from slight initial hypertrophy of the infected nucleus, it is the pressure from within which causes the host nucleus to increase enormously in volume, merely by passive stretching of the nuclear membrane.

The occurrence of successive binary fissions in *Pseudoklossia patellae* (Debaisieux, 1922—though schizogony *sensu stricto* was not observed), and the division into 4 individuals of the merozoites of *Klossia helicina* Schneider (Naville, 1927), suggest the possibility, in *Polydora*, of multiplication of the primary invaders of the nuclei; but this is unlikely, since not only are solitary individuals of different sizes far more common than associated forms, but there is often a discrepancy in the age, size, nature, and number of parasites within the same nucleus, schizonts even being found alongside gametocytes (Fig. 7).

(*b*) *Schizogony.*

Stages in schizogony are relatively very scarce in my material. The youngest forms are subspherical, and measure 2–3μ in diameter (see smaller of the two parasites in Fig. 1). The nucleus contains a small karyosome embedded in a mass of chromatin, and the nuclear membrane is indistinct. Forms destined to become schizonts are recognisable by the peripheral arrangement of the chromatin; this is connected by strands of chromatic material to the karyosome, which is placed eccentrically (see larger of the two parasites in Fig. 1). The karyosome, at all stages, may multiply by budding.

A prospective schizont grows to a considerable size (up to a maximum of $10{\cdot}5 \times 8\mu$) before it shows signs of nuclear division. The nucleus undergoes an atypical mitosis. It becomes spindle-shaped, and extends across the shorter diameter of the cell; and, at anaphase, 3 chromosomes can be counted near each pole (Fig. 2). The karyosomes are ejected into the cytoplasm either at the first nuclear division or during subsequent mitoses (Figs. 2 and 3).

After a number of divisions, the nuclei move to the periphery of the schizont and continue to divide mitotically there, 3 chromosomes appearing on the equatorial plate at each division (Fig. 4). The daughter nuclei show a peripheral arrangement of the chromatin, which is disposed somewhat

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Legends to Figs. 1–6.

Fig. 1. Uninucleate schizont associated with a small, undifferentiated parasite within the same host-nucleus.

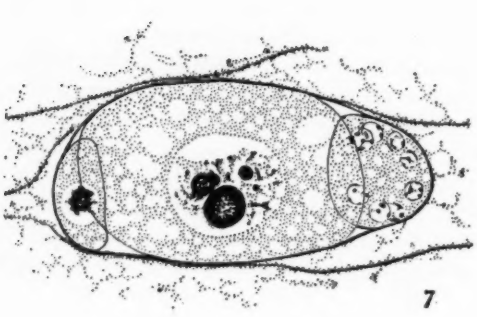
Fig. 2. Schizont: first nuclear division. Three short, rod-like chromosomes are seen passing to each pole (late anaphase). Note the karyosome in the act of being ejected.

Fig. 3. Schizont with 8 nuclei; note the persistent karyosomes, derived from the primary nucleus, lying in the cytoplasm.

Fig. 4. Two multinucleate schizonts inside the same host nucleus; note the mitotic figures showing 3 chromosomes on the equatorial plate.

Fig. 5. Group of merozoites within the persistent nuclear membrane of infected cell.

Fig. 6. Young female parasites lying free in vacuoles inside infected nuclei; note the remains of the chromatin of the latter lining the vacuoles.



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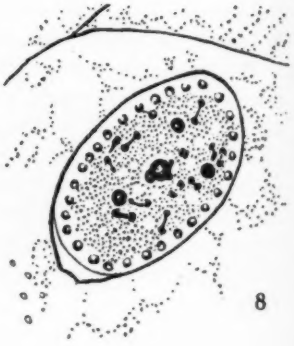

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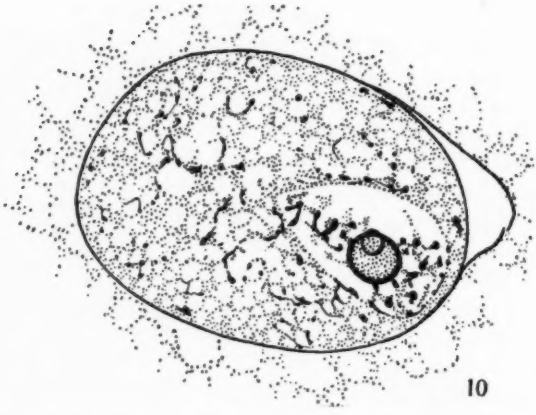

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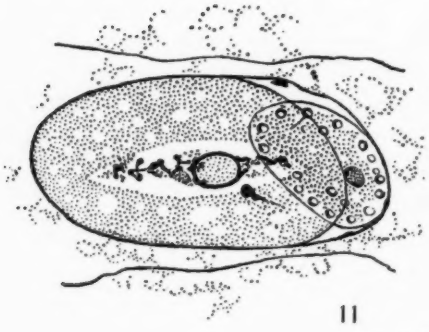

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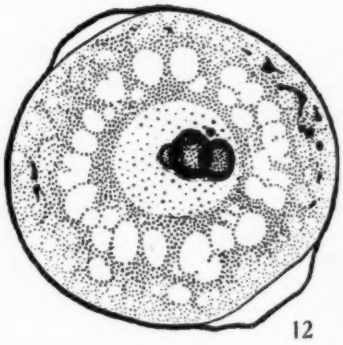

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Figs. 7–12.

irregularly. Mature schizonts vary in size between $8{\cdot}5 \times 8{\cdot}5$ and $15 \times 11{\cdot}5\mu$.

Many merozoites are produced by each schizont, and, even after their formation, the membrane of the host nucleus is still intact (Fig. 5). The merozoites are fusiform bodies, 4μ in length. They lie scattered in groups on the surface of the schizont, with a small cytoplasmic residue in their midst. The nucleus of the merozoite is so small that it appears simply as a solid mass of chromatin.

(*c*) *Microgametogenesis.*

Male parasites are often found associated with females inside the same host nucleus (Fig. 11). This association, and the great inequality in size between the sexes, suggests that the parasite has affinities with the Adeleidea; but the large number of microgametes produced by each male should dispel this impression at once. I believe that the association is accidental and is simply due to the very limited space available for occupation by these strictly intranuclear inhabitants. Moreover, although such an association is frequently met with, solitary females are far more common—male parasites being relatively rare.

I cannot with certainty distinguish males from females when they are very small. I believe that young male parasites are represented by slightly elongated forms (Fig. 7), in which the nucleus occupies almost the whole width of the cell, and contains one or more karyosomes embedded in a mass of chromatin. The structure of the female nucleus is similar. In neither sex is the chromatin arranged peripherally as it is in the schizonts.

Nuclear division begins at a very early stage in microgametocytes, as many as 20 nuclei being present when their dimensions are only $5{\cdot}5 \times 6{\cdot}0\mu$. They can easily be distinguished, therefore, from schizonts, in which most of the growth takes place before the onset of nuclear division. I cannot say how the first nuclei are formed; but in large male parasites (Fig. 8) minute mitotic figures can often be discerned: they are usually in late anaphase. From the very beginning, the nuclei lie just under the very thin pellicle of the organism.

Legends to Figs. 7–12.

Fig. 7. Promiscuous association of individuals within the same nucleus: a schizont, a female parasite, and a uninucleate microgametocyte (?).

Fig. 8. Multinucleate male parasite. Note the vesicular nuclei, the minute spindles and remains of ejected karyosomes.

Fig. 9. Microgametes. Note that the nuclear membrane of the infected cell has disappeared.

Fig. 10. Mature macrogamete, at the time of fertilisation. The nucleus has migrated to the periphery, where it is starting to elongate. The principal karyosome has assumed an ovoid shape.

Fig. 11. Fertilisation spindle. Note the characteristic ovoid karyosome; and, also, remains of the host nuclear chromatin collected in a mass at one point on the enclosing membrane.

Fig. 12. Oocyst. A zone of basophilous cytoplasm, containing large vacuoles, surrounds the nucleus. The peripheral oxyphilous cytoplasm contains dark-staining inclusions of varying size. There is hardly any trace of chromatin in the nucleus, which contains three spherical karyosomes.

At first they closely resemble those of schizonts; but later they appear as clear spherical vesicles with the chromatin concentrated in a crescentic patch on one side. At this stage, the cytoplasm shrinks away from the pellicle, the nuclei remaining attached to it by delicate protoplasmic bridges. The karyosomes derived from the original nucleus are evidently thrown out early, but they persist for a long time as dark-staining patches in the cytoplasm and ultimately disappear.

The crescentic patch of chromatin lining each vesicle gradually assumes the form of a microgamete, becoming drawn out into a slender tail which perhaps represents a posterior flagellum (Fig. 9). No other trace of flagella could be detected. The male parasites vary much in size when mature (10×9–$19 \times 15\mu$), and when the microgametes have been formed, the membrane of the host nucleus has usually disappeared.

The microgametocyte nuclei stain intensely with Feulgen's nucleal reagent; but, oddly enough, all other stages in gametogenesis give negative results. Sassuchin (1935), likewise, found that, in *Eimeria perforans* and *E. faurei*, only the male parasites give a positive reaction. In *Polydora*, this phenomenon is all the more striking, since each parasite or group of parasites appears encircled by a purple ring—the remains of the infected nucleus: thus Feulgen's reagent affords convincing proof of nuclear infection.

(*d*) *Macrogametogenesis.*

The female parasites are by far the commonest forms in my sections, and they attain a much larger size than either males or schizonts. For some time they are completely spherical, but with growth they gradually elongate (Fig. 7) and their cytoplasm acquires a homogeneous vacuolated appearance. The nucleus next migrates to the periphery (Fig. 10) where only an extremely thin layer of cytoplasm now separates it from the pellicle of the parasite. The chromatic material has increased considerably, and has assumed the form of a coarsely beaded reticulum. The principal karyosome is becoming oval.

I believe that fertilisation now occurs; for the nucleus begins to elongate, and continues to do so until it has assumed the form of a fertilisation spindle, which consists of an axial reticulum of beaded chromatin threads and a conspicuous ovoid karyosome (Fig. 11). Macrogametes, at this stage, measure $21{\cdot}5 \times 14$–$35 \times 15\mu$.

The spindle now begins to shorten and a change in the staining reaction of the cytoplasm can be detected. The part adjoining the nucleus becomes more and more basophilous and filled with large vacuoles, the peripheral zone of cytoplasm remaining oxyphilous. This change in the reaction of the cytoplasm is probably connected with fertilisation (cf. Dobell, 1925). Meanwhile the macrogamete has been rounding up, and by the time the fertilisation spindle has contracted up to the surface, it has become spherical, and the large ovoid karyosome has divided into two spherical karyosomes. The nucleus moves to the centre of the zygote; and the differentiation of the cytoplasm

into a circumnuclear basophilous zone containing large vacuoles, and a peripheral oxyphilous zone, becoming increasingly packed with densely staining rods and granules, is now very clear (Fig. 12).

The karyosomes in the female parasites are the largest to be found at any stage in the life cycle, and are therefore most favourable for study. In sections lightly stained with iron haematoxylin, dark granules can be seen lying within the karyosome; and they can also be seen in sections stained with Mann's methyl blue-eosin or safranin and light green. But they show most clearly in those treated with gentian violet and iodine, and strongly differentiated in clove oil until all trace of the stain has been removed from the rest of the nucleus. Most of them lie towards the surface of the karyosome, and one is usually much larger than the rest (Fig. 13).

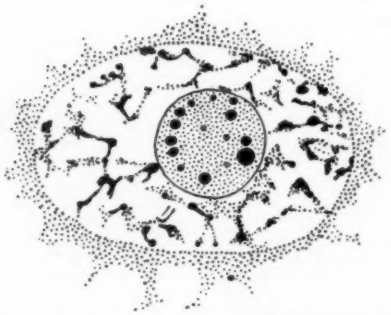
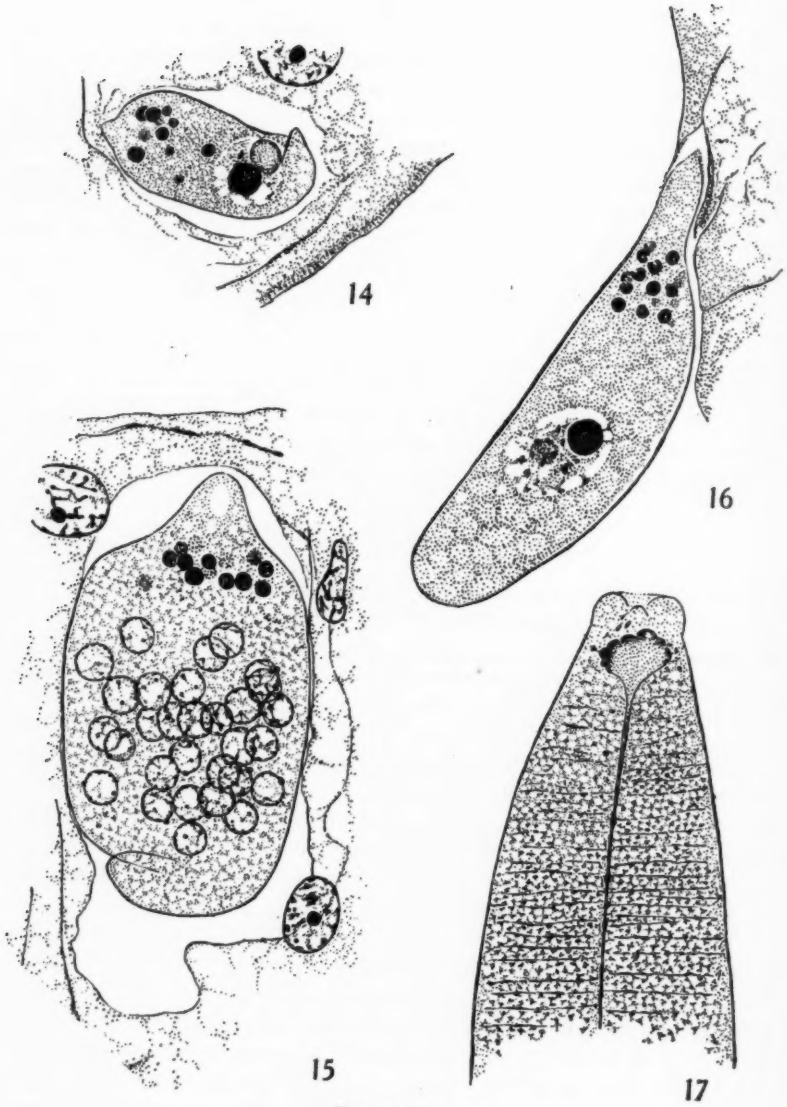


Fig. 13. Coccidian; nucleus of macrogamete showing karyosome with peripheral system of granules (from a section stained with gentian violet and iodine, strongly differentiated). ×4250.

The most mature zygotes (19–26μ diameter) show 2–3 spherical karyosomes of equal size; and the nucleoplasm, which hitherto has remained colourless in stained preparations, now appears pink with chromotrope 2R as counterstain. There seems to be very little chromatic material (Fig. 12). A definite oocyst can now be seen; and later it appears double, consisting of a thick outer and a thin inner layer.

At this stage, the oocysts are passed out of the worm: they probably require the stimulus of sea water in order to complete their development. In cases of heavy infection, the oocysts are liberated *en masse* in faecal strings, which are largely composed of broken-down tissue and mucus: in sections of *Polydora*, such a string appears as a bag-like structure, crammed with zygotes and mature male parasites. I have often seen the contents of the oocysts contract; but although I have kept many for days, or even weeks, under aeration in a moist chamber, I have never been able to make them develop any further. My failure is most unfortunate, since without information as to what results from division of the zygote, it is impossible to refer the coccidian



Figs. 14–17.

from *Polydora flava* to its systematic position. But at least I have described enough to show that we have here a coccidian of eimeriid affinities, and one unlike any hitherto recorded from annelid worms.[1]

(ii) Selenidium axiferens *n.sp.* (=Selenidium *sp. Caullery & Mesnil*)

Until comparatively recently, schizogregarines of the genus *Selenidium* had always been credited with a simple structure. The work of Ray (1930 *a*) and of Mackinnon & Ray (1933) has, however, demonstrated clearly that the bodies of these "nematoid" gregarines show constantly a sufficient number of definite morphological characters to enable one to base specific definitions on these alone.

One of these organisms is common in the gut of *Polydora flava*,[2] and its trophozoites are so remarkable that I therefore feel justified in creating a new species for it, even though I have no knowledge of its spores.

I observed *Selenidium axiferens* n.sp. free in the gut of 60 per cent. of the worms that I examined. Specimens vary in size between 155×14 and $300 \times 31\,\mu$. In some worms extremely narrow forms were found in large numbers, a phenomenon which recalls the dimorphism recorded by Brasil & Fantham (1907) for 2 species of *Selenidium* from *Phascolosoma vulgare* and *P. elongatum*. These two types of *Selenidium* are connected, however, by intermediates, so that the length/breadth ratio is inconstant.

Transverse sections show that the organism is circular in outline throughout its length. The knob-like epimerite (Fig. 18) bears a small hyaline nipple on its anterior surface; and, in fixed specimens, this organ is found in varying states of retraction, a strongly invaginated epimerite giving a truncated appearance to the organism (Fig. 17). At the base of the epimerite lie a number of short siderophilous rods disposed in a circle; owing to their small size it is difficult to demonstrate these bodies.

[1] The only other gut-inhabiting coccidian, from an annelid worm, that has been described in detail is *Dorisiella scolelepidis* Ray (1930 *b*). Other species of Coccidia occurring in annelids include *Caryotropha mesnili* Siedlecki (1907), *Myriospora trophoniae* Lermantoff (1913), *Angeiocystis audouiniae* Brasil (1909), and an aggregatid (see Hoare's classification of the Eimeriidea 1933) from *Nereis diversicolor* (Dehorne, 1930; Thomas, 1930).

[2] Where it was first observed by Caullery & Mesnil (1899), although not given a name.

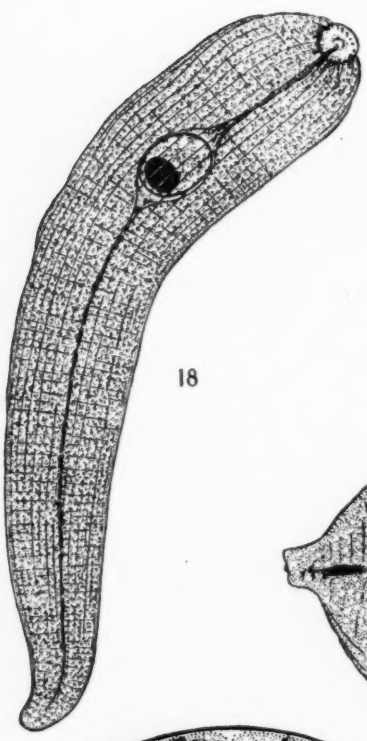
Legends to Figs. 14–17.

Fig. 14. Uninucleate schizont (?), with chromatic globules at the anterior end, 2 karyosomes in the nucleus, and posterior end drawn out into a tail.

Fig. 15. Multinucleate schizont; again note the chromatic globules and the tail. A small extranuclear dot lies in contact with some of the nuclei.

Fig. 16. Young trophozoite (?) lying free in lumen of gut, attached by nipple to epithelium. The nucleus has assumed an oval outline, and one of the karyosomes is in process of disintegration. Note the well-developed cuticle.

Fig. 17. Anterior end of trophozoite, showing funnel-shaped structure formed by fusion of chromatic globules.



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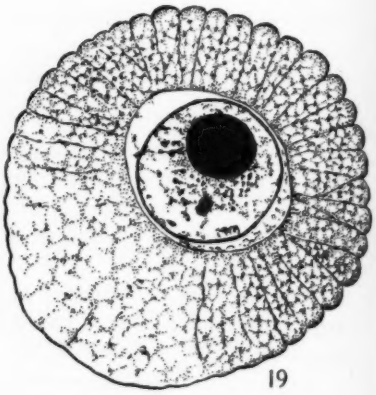

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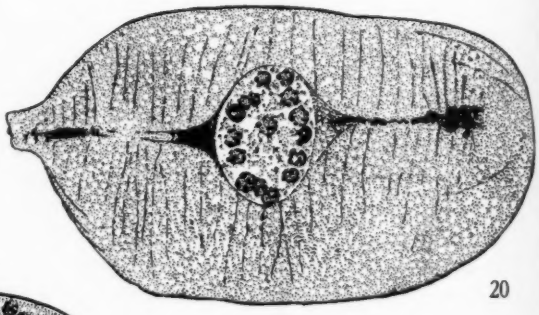

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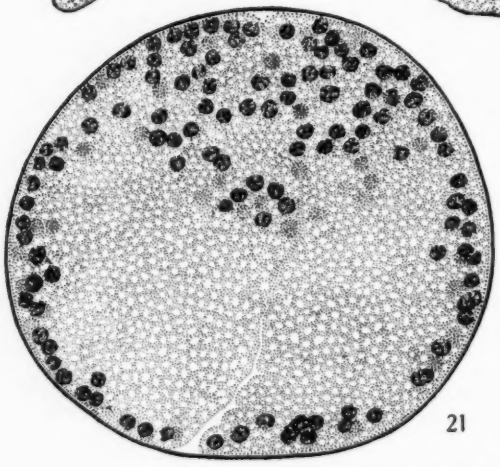

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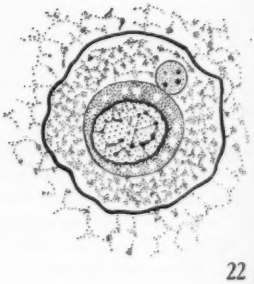

22

Figs. 18–22.

The pellicle is well developed and bears 30–45 longitudinal grooves. The nucleus, which lies about a third of the way along the body, is oval, with its long axis parallel to that of the body. In extremely narrow forms, the nucleus is still more elongate and its shorter diameter occupies nearly the whole width of the organism. A large, slightly bean-shaped karyosome lies at the anterior pole of the nucleus, but occasionally it is found at the posterior end (Fig. 18). The centre of the nucleus is occupied by a diffuse cloud of achromatic material containing a few darker staining granules. It can be shown by Feulgen's reaction that the karyosome is surrounded by a network of chromatin. The rest of the nuclear contents take up only the counterstain (light green): that is to say, most (if not all) of the chromatin is concentrated round the karyosome.

But the peculiar structural feature in this species is the axial tube, which extends from one end of the organism to the other and stains black with iron haematoxylin. This structure was noticed by Caullery & Mesnil (1899), but they alluded to it as a thin plate dividing the body into two halves: a false impression, no doubt, gained from oblique sections. They noticed a similar structure in the species of *Selenidium* from *Pygospio setincornis* Oerst. and *Polydora coeca* Oerst. An axial organ was described by Ray (1930 *a*) for *Selenidium spionis* (Köll.), and Mingazzini, as early as 1891, drew attention to this structure, which is said (Ray) to fade away fore and aft the nucleus. It is interesting to find that, in the type species of the genus, *S. pendula* Giard, a thin layer of hyaline cytoplasm surrounds the nucleus, and extends along the median axis on either side, though tapering off as it does so (Brasil, 1909).

The axial organ of *S. axiferens*, however, is far more conspicuous than anything described before in similar gregarines: it is a tubular rod, containing hyaline cytoplasm, which runs the whole length of the organism, and the nucleus lies within a capsular expansion of it (Figs. 18 and 19). Caullery and Mesnil apparently never saw this capsule, for they remarked that the axial organ "manque à l'endroit du noyau". At its anterior end, the axial tube has a slight spiral twist, and projects into a vacuolated area situated beneath the epimerite.

Legends to Figs. 18–22.

Fig. 18. A full-grown trophozoite. Note the axial tube, running the whole length of the organism, and forming a complete enclosure to the nucleus (capsular expansion of axial tube). (A composite drawing.) × 700.

Fig. 19. Transverse section of trophozoite in region of nucleus, showing radial fibrils connecting the bases of the pellicular grooves to the capsular expansion of the axial tube. × 1650.

Fig. 20. Gametocyte. A number of nuclei have appeared within the nuclear area. × 1050.

Fig. 21. Gametocyst. The nuclei have migrated to the periphery of the cytoplasm where they have undergone further division. × 1050.

Fig. 22. *Polyrhabdina polydorae* Caul. & Mesn. (?). Nucleus showing double zonation of the main karyosome. Note the reticular nature of the inner zone. (From a section stained with Dobell's modification of Mann's methyl blue-eosin.) × 1650.

The axial tube is most conveniently studied in living specimens; for it is very difficult to stain this structure uniformly, unless it is heavily overstained—in which case it appears solid.

At regular intervals, from the base of each pellicular groove, a fibril runs centripetally to be inserted on the axial tube, and in the region of the nucleus these fibrils end on the nuclear capsule (Fig. 19). Longitudinal sections show the zebroid markings so characteristic of the genus, and these are undoubtedly due to the fibrils just mentioned. The significance of these fibrils, and of the axial tube, in relation to the movements of the organism, will be discussed in another paper.

I believe that *S. axiferens* possesses a schizogonic phase in its life cycle, for occasionally I have found intracellular bodies in great abundance. They can easily be distinguished from the coccidian because they are not intranuclear, and they always lie in large well-defined vacuoles in the cytoplasm of the infected cells.

Most of these forms are uninucleate, and they vary in size between $13{\cdot}5 \times 6{\cdot}5$ and $38{\cdot}5 \times 14{\cdot}5\,\mu$; a small specimen is shown in Fig. 14. The nucleus occupies nearly the whole width of the body; a chromatic network fills up the whole nucleus, which typically contains 2 large karyosomes of equal size. At the anterior end of the body lie numerous chromatic globules arranged in 2 or 3 superimposed rings: these characteristic inclusions increase in number with the growth of the parasite. The cytoplasm which they enclose is often vacuolated. The anterior end of the organism is drawn out into a small nipple, which sometimes contains a vacuole; while the posterior end is frequently provided with a tail, which is folded back against the body.

Although it might seem safe to deduce, from the density of the infection, that these forms are the products of a previous schizogony, I should mention that similar multinucleate individuals are not infrequent. These already contain many nuclei (Fig. 15) scattered throughout the cytoplasm; and they show the nipple at the anterior end, the folded tail, and the chromatic globules. They measure 26×20–$35 \times 17\,\mu$. Clearly these are schizonts in process of forming merozoites; but, unfortunately, I have not seen these ultimate products, and I am therefore unable to give convincing evidence for the derivation of the uninucleate forms. Minute granules, lying in the cytoplasm in contact with the nuclei of the dividing schizont, may be the precursors of the chromatic globules. Anyhow, it is quite clear that pre-schizogonic forms contain chromatic globules, unless there is, of course, more than one generation of merozoites; but of this I have not much evidence.

There are certain points of resemblance between these intracellular organisms and the peculiar coccidian *Dorisiella scolelepidis* Ray (1930 *b*); and it is just possible that the worm harbours another species of *Selenidium* akin to *S. mesnili* Brasil from *Myxicola infundibulum* Mont. (Brasil, 1909; Ray, 1930 *a*). However, I favour the view that we have here the asexual phase of *Selenidium axiferens*.

My reasons for this opinion are as follows: some of the uninucleate forms are longer than others, and have a very distinct pellicle; and I have found a few in the lumen of the gut attached to the intestinal epithelium by the nipple (Fig. 16). The nucleus is ovoid, and, in some specimens, its membrane has become more distinct. One of the two karyosomes is disintegrating (Fig. 16), and it will be remembered that the trophozoite of *S. axiferens* has only one. The chromatic globules, at the anterior end, apparently fuse to form a sidero-philous funnel, which becomes drawn out posteriorly along the main axis of the parasite to form the characteristic axial tube of the adult (Fig. 17). The rim of this funnel is surmounted by crescentic structures indicative of its origin from the chromatic globules. Some time elapses before the funnel-shaped structure disappears, to judge from the size of trophozoites which still show it; the short, dark-staining rods, at the base of the epimerite, represent the remains of the funnel.

The sexual phase is very similar to that described for other species of *Selenidium*. The gregarines associate by their posterior ends, and become thicker and shorter. After transverse elongation of their nuclei, small dense nuclei appear within the nuclear area,[1] while it is still enclosed in the capsular expansion of the axial tube (Fig. 20): they appear vesicular and stain a deep purple with Feulgen's reagent, though they appear *in vivo* as clear refringent spherules. The axial tube, with its associated strands, then disappears completely, and the nuclei move out into the peripheral cytoplasm, where they appear to undergo further division (Fig. 21). By the time that each pair of gametocytes has assumed the form of a compact spherical body, a thin gametocyst has been secreted: its dimensions are 48×56–$70 \times 63\,\mu$. The gametes are isogamous; but they are not produced until the cysts have been deposited in sea water, and unfortunately I have not been able to trace sporogony beyond this point.

(iii) Polyrhabdina polydorae *Caul. & Mesn.* (?) (=Lecudina polydorae *Kamm*, 1922)

The details of the morphology of this dicystid gregarine have already been described by Mackinnon & Ray (1931). I have frequently seen it in my sections, and I have a few points to add to their account of the trophozoite.

In sections treated with Mann's methyl blue-eosin, the large karyosome is shown to consist of two sharply delimited zones, the outer being vacuolated, while the inner is reticular (Fig. 22). It can be shown, by Feulgen's method, that the inner zone contains granules of chromatin, while the outer is achromatic; and suitable differentiation of sections stained with Mann's stain confirms this, the outer zone staining pink, the inner blue. This agrees with the

[1] In those species of *Selenidium* in which sporogony has been described, this phase is always initiated by the appearance of daughter nuclei within the nuclear area of the gametocyte. In *S. mesnili*, however, the first mitotic spindle is to be found in the cytoplasm, all trace of the primary nucleus having disappeared (Reed, 1933).

definition, given by Watson (1916), of a karyosome as a chromatic mass surrounded by plastin.

It has been suggested by Mackinnon & Ray that these dicystids are not intracellular at any stage. In support of this contention, I may state that the youngest forms that I have ever discovered measure $25 \times 12{\cdot}5\,\mu$ (the adults measure $100\text{–}180 \times 30\text{–}40\,\mu$ according to the above authors), and these are definitely extracellular. The epimerite[1] is relatively much larger at this stage, and is clearly sunk in a depression of the surface of an epithelial cell.

I have succeeded in finding associating individuals and gametocysts of this gregarine. A thick, striated gelatinous cyst is formed round the gametocytes while they are still uninucleate; and it seems probable that the cyst is secreted in the form of gelatinous threads in a manner similar to that described for *Leidyana erratica* Crawley (Watson, 1916). Early stages of nuclear division I have not seen; but, in a number of my sections, innumerable nuclei have already appeared, and they lie scattered throughout the cytoplasm. These nuclei stain rather faintly compared with those in the gametocysts of *Selenidium axiferens*; each nucleus contains two small chromatic granules side by side. The pellicle is so remarkably persistent that, even at this stage, its characteristic striations can be clearly discerned. The gametocysts are larger than those of *Selenidium*: they measure $98 \times 90\text{–}105 \times 100\,\mu$.

Here again, as with some other dicystids from marine worms (Mackinnon & Ray, 1931), the cysts probably require the stimulus of sea water in order to complete their development.

IV. Summary

1. A detailed description is given of a coccidian which parasitises the nuclei of the intestinal cells of the polychaete worm *Polydora flava* Claparède. The final stages in sporogony have not been observed; nevertheless, this is the first record, from an annelid worm, of an eimeriid which shows a striking resemblance to those Coccidia occurring in the higher animals. It is also the first record of an intranuclear parasite from an annelid.

2. From the gut of the same worm, a new species of *Selenidium* is described. It differs from all other species, hitherto recorded, in the possession of an axial tube, which completely encloses the nucleus and extends from one end of the organism to the other: hence it is proposed to name this organism *Selenidium axiferens*. The anterior end of the trophozoite shows a circlet of siderophilous bodies, which Ray (1930 *a*) has shown to be characteristic of the genus.

3. Both uninucleate and multinucleate intracellular forms, with polar groups of chromatic globules, have been discovered in great abundance on six occasions; no merozoites have been found, but evidence is submitted to

[1] The epimeritic collar, and the ring of prongs just anterior to it, are well developed at this stage; they both stain pink with Mann's stain.

show that these intracellular forms represent the schizogonic phase in the life-cycle of the *Selenidium*.

4. Sporogony follows closely that described for other species, but the final stages have not been observed. The gametocysts measure 48×50–$70 \times 63\,\mu$.

5. Association and cyst formation are described in *Polyrhabdina polydorae* Caul. & Mesn. (?) for the first time. Innumerable nuclei make their appearance in each gametocyte, before ejection of the cysts into the sea-water takes place. Gametocysts can easily be distinguished from those of *Selenidium axiferens*, not only by their morphology, but also by their greater size (98×90–$105 \times 100\,\mu$).

For her expert advice, and helpful criticism, my sincerest thanks are due to Prof. Doris L. Mackinnon, who suggested this work and under whose direction it has been carried out. I should also like to thank Dr H. N. Ray for the preparations that he left behind in the Department; without the aid of these, I should not have been able to give such a complete account of the coccidian. For the kindness and hospitality extended to me during my visits to Plymouth, I should like to express my gratitude to Dr E. J. Allen, F.R.S., and other members of the Marine Biological Association at Plymouth. I am particularly grateful to Mr D. A. Kempson for the valuable assistance that he has rendered me in the laboratory at King's College. The work was carried out during my tenure of the Harold Row Research Studentship at King's College.

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STUDIES ON THE INFLUENCE OF THE ENVIRONMENT ON THE SHEEP BLOW-FLY *LUCILIA SERICATA* MEIG.

IV. THE INDIRECT EFFECT OF TEMPERATURE AND HUMIDITY ACTING THROUGH CERTAIN COMPETING SPECIES OF BLOW-FLIES

By A. C. EVANS

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(With 2 Figures in the Text)

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I. Introduction

In the former papers of this series, Evans (1934, 1935, 1935 *a*, 1935 *b*), the direct effect of temperature and humidity on *Lucilia sericata* were studied. In considering the effects of climate upon one species of an ecological complex, it is clearly necessary to understand its effects upon the associated species. This is especially so in the case of blow-flies, where competition plays such an important part in determining the abundance of the different species. Holdaway (1930) records that only 231 individuals of *L. sericata* out of an initial population of approximately 50,000 reached the adult stage, this huge mortality being due chiefly to dipterous competition. In the present paper consideration is given to the effects of temperature and humidity in determining the survival and development of some of the principal species of English blow-flies which compete with *L. sericata*, namely, *Calliphora erythrocephala* Meig., *Phormia groenlandica* Zett., and *Sarcophaga falculata* Pand. These three species together with *Lucilia sericata* are typical examples of the blow-fly fauna of Great Britain and have been studied to some extent by Graham-Smith (1916) and Wardle (1927). The work of these two authors has established that a definite seasonal succession of blow-flies occurs, species of the genus *Calliphora* appearing about a month before and disappearing about a month after the genera *Lucilia* and *Sarcophaga*. The position of the genus *Phormia* in the succession is not yet quite clear on account of the small

numbers taken, but it seems on the whole to be after that of *Lucilia* and *Sarcophaga*. The author's own observations in an insectary on the roof of the London School of Hygiene and Tropical Medicine also support this view. Graham-Smith (1916) suggests that the time of the spring emergence is dependent on a rise of the ground temperature to a critical point for each species and suggests a temperature of 9–10° C. for *Calliphora erythrocephala*. Wardle (1927) concurs, in the main, with this viewpoint for *C. erythrocephala*, stating that the appearance of this species coincided with a rise of the mean daily minimum temperature above 5° C., but that the appearance of *Lucilia* and *Phormia* seemed independent of a rise in the mean daily minimum temperature above any critical point. In the case of *Lucilia* the lack of any relationship between the time of appearance and a rise in the mean daily minimum temperature above a definite temperature may be understood in view of the work of Cousin (1932). Cousin showed that low temperatures and, in general, adverse environmental conditions bring about a condition of diapause in the prepupal stage, in which stage *L. sericata* hibernates (Holdaway & Evans, 1930; Davies, 1930), and also that the intensity of the stimulus which brings the diapause to an end and permits of pupation is in direct relation with the intensity of the adverse conditions causing the diapause. Thus, in the spring, the mean daily minimum temperature necessary to break the diapause conditions of the hibernating larvae may depend on the mildness or severity of the preceding autumn and winter, a low mean daily minimum temperature will follow a mild winter and a high mean daily minimum will follow a severe winter. However, it must be noted that Graham-Smith (1916) records *L. sericata*, amongst other flies, as passing the winter as pupae or less commonly as larvae, in the earth. If it is true that *L. sericata* can hibernate in the pupal as well as in the prepupal stage, then it may well be that the first appearance of *L. sericata* is dependent on a rise of the ground temperature to a definite critical point governed by the threshold temperature. Further observations are required to establish definitely in which stage the various species of blow-flies hibernate, and also whether the climatic conditions prevailing during late autumn have any effect in determining the stage in which hibernation occurs.

Phormia groenlandica hibernates in the adult stage (Roubaud, 1927). Nicholson (1934) has presented some interesting comparative data on the effect of temperature on the activity of various species of blow-flies. The lowest temperature at which the flies are active enough to seek food varies of course with the temperature and lies between 17 and 20° C., which is much higher than the threshold temperature for the metamorphic stage given by Peairs (1927) as 6–7° C. for similar flies. This difference between the threshold temperature for development and the lowest temperature for adult activity might explain why *P. groenlandica* appears last in the seasonal succession.

The data discussed below show that, in their resistance to high temperature and low humidities, the four species studied can be arranged in the opposite order to their seasonal succession, that is to say, the earliest to appear is least

resistant and the last to appear the most resistant to high temperature and to low humidity. Thus the reasons for their degree of resistance may well be allied to or the same as those which determine their seasonal succession.

The methods of study and numbered individuals used were the same as described in the previous papers of this series.

II. THE RATE OF DEVELOPMENT OF THE COMPETING SPECIES

Table I shows the times of development in hours at 25 and at 33° C. of the egg, prepupa and pupa in the four species studied. *Sarcophaga falculata* deposits living larvae and no data are available as to the times of development of its eggs.

Table I. *The effect of temperature on time of development expressed in hours at* 25 *and* 33° C.

Temp. (° C.)	*Lucilia*	*Calliphora*	*Sarcophaga*	*Phormia*
		Egg		
25	14	14	—	19
33	9	13	—	11
		Prepupa		
25	72	72	144	40
33	43	All dead	96	30
		Pupa		
25	174	192	214	100
33	116	All dead	208	142

There is not a great deal of difference in the times taken by the eggs of the various species to complete their development at each temperature, but those of *Phormia groenlandica* develop more slowly than those of *Lucilia sericata*. At 25° C. the times of development for *L. sericata* and *Calliphora erythrocephala* are the same, but at 33° C. the eggs of *Lucilia sericata* develop much more rapidly than those of *Calliphora erythrocephala*. In view of the work of Prochnow (1908) on the influence of temperature on rate of development and the fact that a temperature of 33° C. is lethal for the prepupal and pupal stages of *C. erythrocephala*, it is possible that this temperature has a retarding effect on the rate of development of the egg of this species rather than that the rate of development is constant from 25 to 33° C.; the maximum rate of development probably occurs at about 30° C.

The times of development given in Table I were all recorded at 100 per cent. relative humidity. The influence of humidity on the rate of development of the egg of *Lucilia sericata* was discussed by Evans (1934), and a linear relationship was shown to hold between the two variables. In the case of *Calliphora erythrocephala* the relationship between time of development and humidity is parabolic. Table II shows the recorded times and the times calculated from the formula

$$y = 0{\cdot}046\,(x + 1{\cdot}19)^2 + 19{\cdot}8,$$

when y = time of development in hours and x = saturation deficiency expressed in mm. Hg.

Table II. *The relationship between time of development expressed in hours of eggs of* Calliphora erythrocephala *and saturation deficiency at* 22° C.

Time of development in hours		Saturation deficiency in mm. Hg
Found	Calculated	
19·9	19·9	0
20·3	20·3	2
21·1	21·1	4
22·2	22·2	6
23·7	23·7	8

In the case of the prepupae, *Phormia groenlandica* develops the most rapidly, then *Lucilia sericata* and *Calliphora erythrocephala*, while *Sarcophaga falculata* develops very slowly. The rates of development for the pupae lie in the same order. It is of interest to note that Peairs (1927) has recorded the time of development of a sarcophagid doubtfully identified as *S. carnaria*. The times of development at 25 and 33° C. are very similar to those recorded above for *Lucilia sericata*, but are very different from those recorded for *Sarcophaga falculata*. *S. carnaria* belongs to that group of sarcophagids possessing black genitalia and *S. falculata* to the group possessing red genitalia. It would be interesting to discover whether this difference in the rates of development held for other members of the two groups.

III. The effect of temperature and humidity on the survival and development of the embryonic, prepupal and pupal stages

Table III shows the percentage survival and development obtained in the eggs of the three oviparous species at 25 and 33° C. over a complete range of humidities.

Table III. *Percentage survival and development of the eggs.* A *at* 25° C. *and* B *at* 33° C.

	Survival						Development					
Relative humidity ...	100	80	60	40	20	0	100	80	60	40	20	0
A. *L. sericata*	95	96	73	0	0	0	98	96	96	62	3	0
P. groenlandica	96	96	34	0	0	0	98	98	92	76	6	0
C. erythrocephala	98	98	76	0	0	0	100	98	86	2	0	0
B. *L. sericata*	96	89	57	0	0	0	98	91	96	49	0	0
P. groenlandica	72	50	10	0	0	0	78	58	28	0	0	0
C. erythrocephala	92	54	0	0	0	0	92	74	30	0	0	0

At 25° C. there is but little difference in the percentage survival of the three species, but the percentage development of *Calliphora erythrocephala* is definitely lower at the lower humidities than that of *Lucilia sericata* and *Phormia groenlandica*. At 33° C. a higher percentage survival and development is obtained from *Lucilia sericata* than from the other two species. The

high mortality is clearly due to humidity in the case of *Calliphora erythrocephala* and *Lucilia sericata*, since nearly a 100 per cent. survival and development is obtained in a saturated atmosphere; in the case of *Phormia groenlandica* it is probably due to a combined effect of temperature and humidity. The higher mortality of the eggs of *Calliphora erythrocephala* and *Phormia groenlandica* compared with that of *Lucilia sericata* due to humidity is brought about by their slower rate of development and consequently longer exposure to the desiccating effect of the dry air.

The susceptibility of *Calliphora erythrocephala* to dry air is clearly brought out in a study of the prepupae and pupae, even at 25° C. there is an appreciable mortality of the prepupae of this species at 20 and at 0 per cent. relative humidity, whereas the prepupae of the other species survive 100 per cent. at all humidities (Table IV).

Table IV. *Percentage pupation of the prepupae*, A *at* 25° C., B *at* 33° C.

Relative humidity ...	100	80	60	40	20	0
A. *L. sericata*	100	100	100	100	100	100
P. groenlandica	100	100	100	100	100	100
C. erythrocephala	100	100	100	100	20	44
S. falculata	100	100	100	100	100	100
B. *L. sericata*	100	100	90	70	40	0
P. groenlandica	100	100	100	100	100	88
C. erythrocephala	0	0	0	0	0	0
S. falculata	84	100	100	100	100	100

A temperature of 33° C. kills all the prepupae of *Calliphora erythrocephala* subjected to it, while it has no effect on the prepupae of the other species (*vide* 100 per cent. survival at 80 per cent. relative humidity). The mortality of the prepupae of these species is clearly brought about by humidity. A saturated atmosphere is deleterious to *Sarcophaga falculata* but does not affect *Lucilia sericata* and *Phormia groenlandica*, the mortality in these species being caused by a too great loss of water at the lower humidities.

The effect of temperature and humidity on the pupae of the four species is substantially the same as on the prepupae (Table V).

Table V. *Percentage survival and development of the pupae.*
A *at* 25° C., B *at* 33° C.

	Survival						Development					
Relative humidity ...	100	80	60	40	20	0	100	80	60	40	20	0
A. *L. sericata*	98	98	98	94	88	80	100	100	100	100	100	98
P. groenlandica	98	98	98	100	84	90	98	100	100	100	100	100
C. erythrocephala	88	98	92	98	64	30	96	100	96	100	94	98
S. falculata	22	100	100	100	90	86	34	100	100	100	100	100
B. *L. sericata*	52	72	50	14	0	0	92	100	98	98	96	94
P. groenlandica	94	96	94	80	80	60	94	96	100	98	100	96
C. erythrocephala	0	0	0	0	0	0	0	0	0	0	0	0
S. falculata	0	76	60	48	40	28	0	100	100	100	100	100

The chief points of interest in Table V concern *Sarcophaga falculata* they are, the complete suppression of development of the pupae in a saturated

atmosphere at 33° C., the low percentage survival and development occurring in a saturated atmosphere at 25° C., and the high mortality at most humidities brought about by a failure to emerge, often because the head of the imago inside the puparium did not quite reach the posterior border of the puparial cap. Consequently the imago could not extend itself sufficiently to use its powerful ptilimum.

If the effect of high temperature and variable humidity on the whole metamorphic period, *i.e.* from cessation of feeding of the larvae to emergence of the adults, is studied, it is found that *Phormia groenlandica* is most resistant to extreme conditions with the other species arranging themselves in the following order, *Sarcophaga falculata*, *Lucilia sericata* and *Calliphora erythrocephala* (Table VI).

Table VI. *The effect of variable humidity on the percentage survival and development of prepupae and pupae maintained continuously at* 33° C.

	Survival						Development					
Relative humidity...	100	80	60	40	20	0	100	80	60	40	20	0
L. sericata	0	4	0	2	2	0	48	62	36	34	22	0
P. groenlandica	88	100	100	96	76	56	96	100	100	100	88	80
C. erythrocephala	0	0	0	0	0	0	0	0	0	0	0	0
S. falculata	4	96	76	52	32	8	4	100	100	84	84	32

IV. The effect of humidity on the length of life of the adult

The length of life of the adults of the four species was studied at 24° C. at 100, 80, 40 and 0 per cent. relative humidities. The flies were fed for 5 days on a complete growing diet of meat, sugar and water. It was intended to feed them until *Lucilia sericata* had commenced to lay eggs, but *Phormia groenlandica* unexpectedly laid eggs first and so the experiment was commenced at this point. Subsequent feeding of the flies not used for the experiment showed that *Lucilia sericata* and *Calliphora erythrocephala* required 2 more days and *Sarcophaga falculata* 7 more days, this latter figure including, of course, the developmental period of the inter-uterine eggs, to reach maturity. The results obtained are shown in Figs. 1 and 2. The most interesting point brought out in these graphs is the difference in shape of the curves for mature (*Phormia groenlandica*) and immature flies (*Lucilia sericata*, *Calliphora erythrocephala* and *Sarcophaga falculata*). A similar difference was shown by Evans (1935) for adults of *Lucilia sericata* fed for 1 day and for 6 days (eggs having been laid on the sixth day). The reason for this difference in the shape of the curves for young and older flies is obscure. That the difference is correlated with sexual maturity seems to be unlikely, as males produce spermatozoa some time before the females lay eggs, yet both sexes show the change at about the same time, *i.e.* the seventh day. Perhaps there is an "ageing" factor which is responsible.

That an "ageing" factor does play a part in the differing length of life of males and females subjected to various humidities is suggested by Table VI.

Table VI. *The length of life of flies* (Lucilia sericata) *of different ages at various humidities* (*flies fed on a complete diet*) 23–24° C.

	Males				Females			
Relative humidity ...	0	40	80	100	0	40	80	100
Age in days								
1	25	63	88	91	27	68	81	96
5	23	30	36	40	21	53	66	68
7	12	12	12	17	28	35	41	39

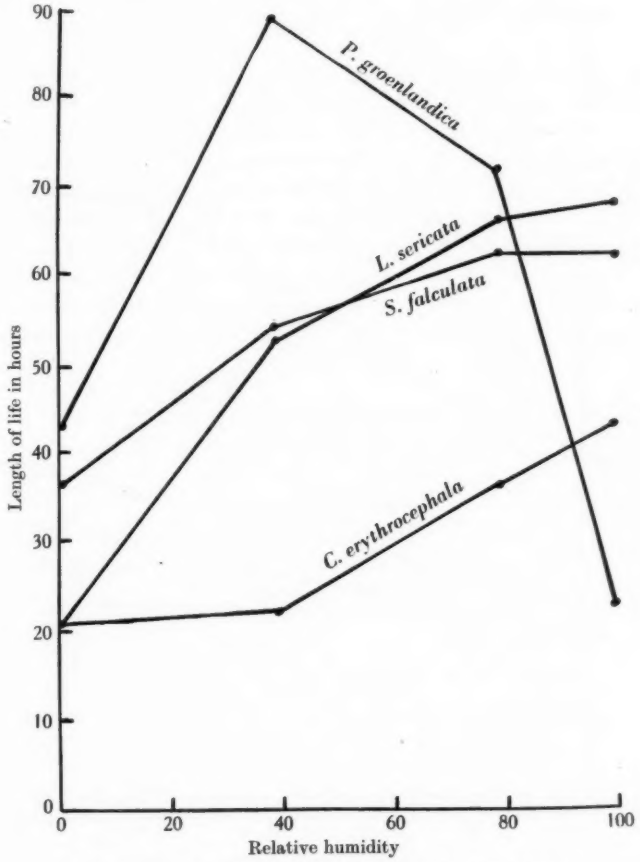

Fig. 1. Length of life of female flies at various humidities.

Thus, although the length of life of young males and females is the same at the same humidity, it will be noticed that the older males live distinctly shorter lives than the older females at the same humidity. Evans (1935) showed that males fed on a complete diet at 23° C. increased in weight rapidly for 2 days and then lost weight fairly rapidly, finally dying somewhat below

their original weight at emergence. The females, on the contrary, reached a maximum weight on the seventh day and then remained at about this weight for 10 days before beginning to lose weight. The weighings were performed on groups of twenty flies, so that fluctuations in weight due to egg laying by a few individuals were about balanced by the increase in weight of other individuals. These facts, together with the results given in Table VI, would suggest that the "ageing" factor is felt earlier and more strongly in the male.

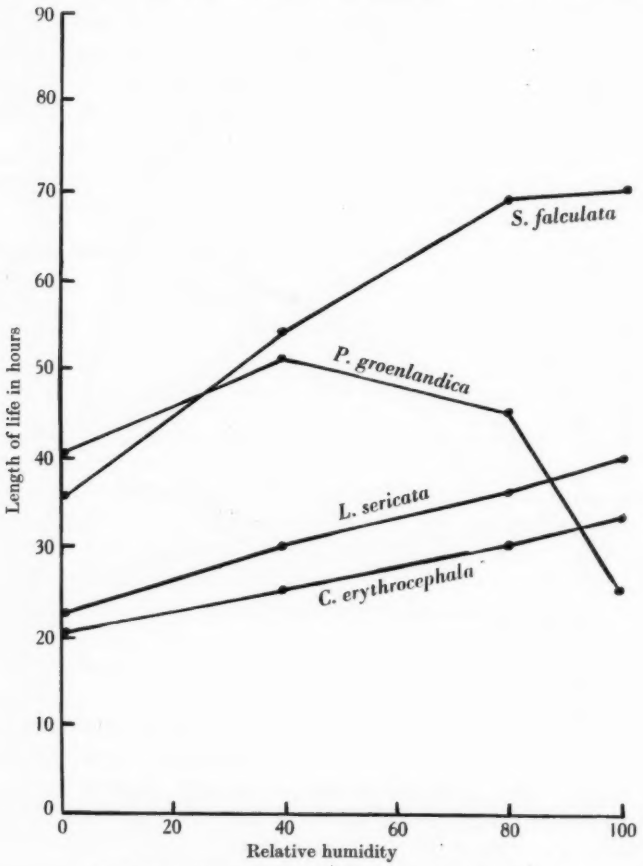

Fig. 2. Length of life of male flies at various humidities.

Although the degree of maturity of the females differs rather in each of the species used in this experiment, it is permissible to draw the conclusion that the females of *Phormia groenlandica* are most resistant to low humidity, the females of *Calliphora erythrocephala* least resistant, and those of *Lucilia sericata* and *Sarcophaga falculata* intermediate in resistance, since Evans (1935)

showed that the length of life of mature females at median humidities is about half that of females fed for 1 day only. Thus immature females of *Phormia groenlandica* would be expected to live even longer than the 90 hours recorded for the mature females. In the case of the males the order of resistance is somewhat different, *Sarcophaga falculata* heading the list followed by *Phormia groenlandica* and *Lucilia sericata*, with *Calliphora erythrocephala* taking its accustomed position at the bottom.

V. SUMMARY

The comparative resistance to high temperature and various humidities of four species of blow-flies, *Calliphora erythrocephala* Meig., *Lucilia sericata* Meig., *Sarcophaga falculata* Pand. and *Phormia groenlandica* Zett. is studied. It is shown that *Phormia groenlandica* is the most resistant species and *Calliphora erythrocephala* the least resistant species, with *Lucilia sericata* and *Sarcophaga falculata* occypying an intermediate position.

This order of resistance is correlated negatively with the order of the seasonal succession of the species.

It is suggested that certain differences in the length of life of males and females subjected to various humidities is brought about by an "ageing" factor.

I wish here to record my great indebtedness to Prof. P. A. Buxton in whose laboratories this work was carried out.

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PHILONEMA AGUBERNACULUM SP.NOV. (DRACUNCULIDAE), A NEMATODE FROM THE BODY CAVITY OF FISHES[1]

By JAMES R. SIMON and FELIX SIMON
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(With 5 Figures in the Text)

The nematode described here was collected by the Wyoming field party of the United States Bureau of Fisheries during the summer of 1934. The Rocky Mountain whitefish, the Eastern Brook trout, and the rainbow trout serve as hosts for the parasite. In several instances pathological changes, particularly of the gonads, were found to accompany its presence.

Forty-four specimens, 16 males and 28 females, were used as the basis for this study. This nematode belongs to the genus *Philonema* Kiutunen-Ekbaum, 1933, and is described as a new species.

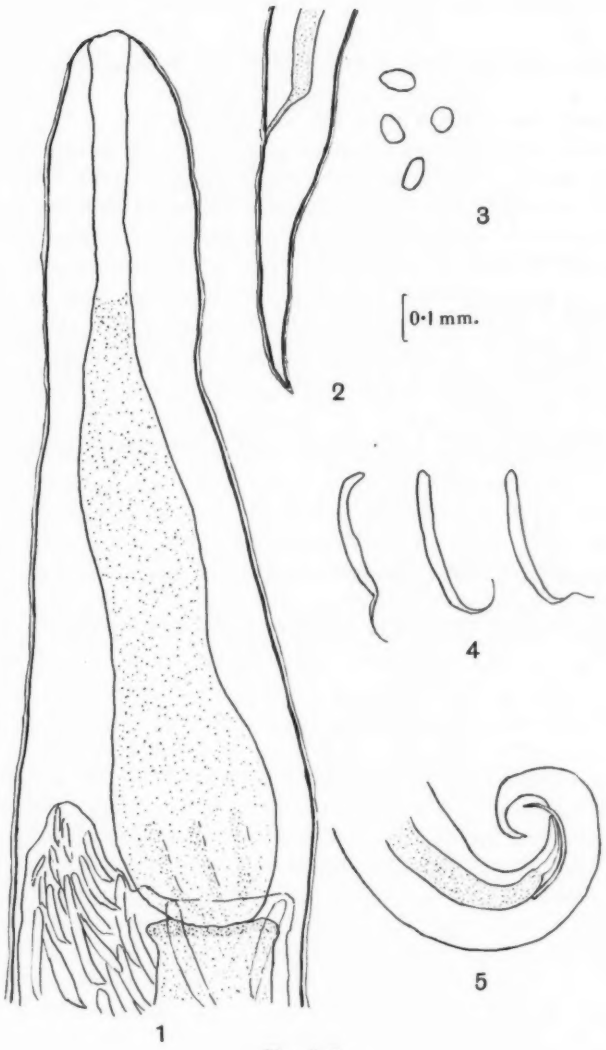
Philonema agubernaculum sp.nov.

Description: *Philonema*. Male: Length 12–23 mm., average 16·5 mm. Maximum width 0·296 mm., well forward. The musculo-glandular oesophagus is cylindrical, averaging 1·526 mm. in length, with an average maximum diameter of 0·189 mm. The anterior, that is, the muscular, portion of the oesophagus measures about 0·404 mm. in length. Anus to end of tail averages 0·276 mm. Spicules equal, sharply curved, lightly chitinised, averaging 0·284 mm. over the curve. Gubernaculum absent. Female: Length 49–140 mm., average 86·1 mm. Average maximum width 0·771 mm. The oesophagus averages 1·725 mm. in length, with an average maximum diameter of 0·220 mm. The anterior part of the oesophagus averages 0·553 mm. in length. Anus to end of tail averages 0·446 mm. Anus sometimes atrophied in gravid females. Vulva and vagina atrophied in gravid females, no vestiges discernible. A small ovary at each end. In gravid females the uterus, filled with embryos, occupies nearly all of the body cavity. Eggs in various stages of development are found in the uteri of young worms. The eggs measure 0·032 by 0·046 mm. Embryos measure 0·343 mm. in length.

Hosts: *Prosopium williamsoni* (Girard). *Salvelinus fontinalis* (Mitchill), and *Salmo shasta* (Jordan).

Location: Body cavity; muscle of abdominal wall.

[1] Published by permission of the United States Commissioner of Fisheries.



Figs. 1–5.

Fig. 1. Anterior extremity female.
Fig. 2. Posterior extremity female.
Fig. 3. Intrauterine ova.
Fig. 4. Intrauterine embryos.
Fig. 5. Posterior extremity male.

Locality: The following waters of the Wyoming National Forest, Wyoming: Green River Lakes, June 15, 1934; Mill Creek, June 26, 1934; Fremont Lake, August 9, 1934.

Disposition of type form: U.S.N.M. Helm. Coll. No. 8908, 2 males and 2 females.

Paratypes: University of Wyoming Parasit. Coll.

Until now there has been described only one other species of the genus *Philonema*, namely, *P. oncorhynchi* Kuitunen-Ekbaum, 1933, from the sockeye salmon, *Oncorhynchus nerka* (Walbaum). *Philonema agubernaculum* differs from *P. oncorhynchi* chiefly by its much smaller size, the females of *P. oncorhynchi* being 2·5 times as long as the females of *P. agubernaculum*, while the males are 1·5 times as long. The ratio in length between male and female of *P. oncorhynchi* is 1 : 10, and between male and female of *P. agubernaculum* 1:6.

A redescription of *P. oncorhynchi* by Smedley (1933) gives the ratio between anterior and posterior parts of the œsophagus as 1 : 1·1. *P. agubernaculum* shows the ratio 1 : 2·7.

The above differences, it is believed, set *P. agubernaculum* aside as a new species.

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FURTHER OBSERVATIONS UPON A "*TRICHOMONAS*" FROM POND WATER

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From the Molteno Institute for Biology and Parasitology

In July, 1934, I found a trichomonad-like organism in water taken from a pond in Lincolnshire. Its morphology, and method of division, and also the cultural conditions under which it will grow, have been described previously (Bishop, 1935). It was named, provisionally, "*Trichomonas*" *keilini*. In "*T.*" *keilini*, unlike most trichomonads, the undulating membrane is short, the bordering flagellum never becomes free, and there is no basal fibre beneath the undulating membrane. "*T.*" *keilini* was never found directly in the pond water but only after it was centrifuged and allowed to stand for 7–21 days.

The question of the relationship of this trichomonad-like form from pond water to the true trichomonads parasitic in animal hosts is of great interest. The possibility that "*T.*" *keilini* is a free-living form incapable of leading a parasitic life, or that it is a parasite of some cold-blooded host (its inability to live in culture at 37° C. (Bishop, 1935) would appear to rule out a warm-blooded host) but capable of living also in pond water, must both be considered. No organism having similar morphological characters has been described hitherto from any host.

Experiments were carried out to discover whether "*T.*" *keilini* could live in frog tadpoles which had been reared free from other Protozoa. There was, of course, no suspicion that frog tadpoles were the natural hosts of this organism. Many workers have studied the intestinal Protozoa of frogs and toads and their larvae, but no flagellate bearing the characteristics of "*T.*" *keilini* has been described from them. But if "*T.*" *keilini* could live in such a host under experimental conditions support would be given to the possibility of its parasitic origin.

On 13. iii. 35, therefore, eggs from a frog were fertilised in pond water sterilised by filtration through a Seitz filter, with all the precautions against contamination described in a previous paper (Bishop, 1934). The eggs hatched, and the resulting tadpoles were fed upon boiled dog biscuit and upon faeces from a person who, during the previous 6½ years, had been examined frequently for intestinal Protozoa, both by direct microscopic examination of faeces and of cultures made from it, with invariably negative results. On 22. iii. 35 eight of these tadpoles were killed, and the whole contents of the gut of each tadpole was examined. Each tadpole was negative for Protozoa.

First series. On 25. iii. 35 six tadpoles were fed upon a strain (Ta.) of "*Trichomonas*" from the pond. This strain was isolated on 10. viii. 34 and grown upon a medium consisting of an inspissated horse-serum slope covered with inactivated horse serum diluted 1 : 10 in 0·5 NaCl solution. It was in its 20th generation, and at no period had cysts been found in it. Six other tadpoles were fed upon a strain (Tb.) of *T. batrachorum* from a toad (*Bufo vulgaris*). This strain had been isolated on 8. x. 34 and was growing on a medium similar to that described above. It was in its 15th generation. Cysts had never been observed in it. Six tadpoles were kept as controls and fed only upon boiled dog biscuit and human faeces.

Second series. On 26. iii. 35 another series of experiments, similar in every particular to those described above, was started, six tadpoles being fed with the Ta strain of "*T.*" *keilini*, six with the Tb strain of *T. batrachorum*, and six being kept as controls.

On 27. iii. 35 one tadpole of the first series fed with "*T.*" *keilini* was killed and its intestinal contents teased up and examined. It was negative for Protozoa. A tadpole of the same series but of the set fed with *T. batrachorum* was similarly examined and found to be infected with that flagellate. On 29. iii. 35 two tadpoles of the second series, one fed with "*T.*" *keilini* and one with *T. batrachorum*, were killed and examined. The former was negative for "*T.*" *keilini* and the latter positive for *T. batrachorum*. On 30. iii. 35 one tadpole of the first series and one of the second series, each fed with "*T.*" *keilini*, were killed and examined and both were negative; whereas one tadpole of the first series and one of the second series, each fed with *T. batrachorum*, were each positive for that flagellate. On 1. iv. 35 two tadpoles, one from each series, fed with "*T.*" *keilini* were both negative; whereas two tadpoles, one from each series, fed with *T. batrachorum* contained that flagellate in their intestines. A control tadpole of the first series was negative. On 2. iv. 35 two more controls were negative as were two tadpoles which had been fed with "*T.*" *keilini*. One tadpole from each series, fed with *T. batrachorum*, was positive. On 3. iv. 35 and 4. iv. 35 similar results were obtained from the remaining tadpoles. The controls were all negative.

That the method used for these feeding experiments was such that infection with *Trichomonas* was mechanically possible was proved by the results from the experiments with *T. batrachorum*, when every tadpole fed became positive. It is evident therefore that the fact that no tadpole became infected with "*T.*" *keilini* was due to the inability of this flagellate to live in the tadpoles and not to any fault in the technique employed.

On 16. viii. 35 I visited the pond again. It was a period of severe drought and many of the ponds in the neighbourhood were dried up. The water in this particular pond was lower than in the previous year, and very concentrated. Two glass containers, previously sterilised, were filled with water from the pond. A few individuals of "*T.*" *keilini* were found in the sediment in one jar. The following day the contents of both vessels were centrifuged at 2000 revolu-

tions per minute for 10 min. The "trichomonads" were numerous in the centrifuged sediment. The sediment, together with some of the supernatant fluid, was put into three sterile tubes and kept at room temperature for 2 days, when the growth of "*Trichomonas*" was dense.

A strain of this "*Trichomonas*" was cultivated in a medium consisting of an inspissated horse-serum slope covered with inactivated horse serum diluted 1 : 10 in 0·5 NaCl solution. A little solid, sterile rice starch was added. This strain was cultivated for 2½ months, through five generations, and abandoned voluntarily. Preparations of individuals from these cultures showed the same morphological characters as "*Trichomonas*" *keilini*.

In the previous paper (Bishop, 1935) the question whether the free form of "*Trichomonas*" *keilini* or only an encysted stage was present in the pond was discussed. The presence recorded above of a few individuals in the normal sediment of the pond water, and of many individuals in the centrifuged sediment within 24 hours of collecting the water, indicates that the free flagellate lives in the pond. Whether "*T.*" *keilini* represents an adaptation of a parasitic *Trichomonas* to a free-living mode of life, or whether the genus *Trichomonas*, of which all the members hitherto described are parasitic, has arisen from a free-living stock of which "*T.*" *keilini* is a survival cannot be decided.

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TENUISENTIS, A NEW GENUS OF ACANTHOCEPHALA, AND ITS TAXONOMIC POSITION[1]

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(With 4 Figures in the Text)

A species of Acanthocephala from *Heterotis niloticus* was described by Meyer (1932, p. 54) as *Rhadinorhynchus niloticus*. Two male individuals from the Egyptian Nile which served as the basis for Meyer's description of this species are deposited in the Berlin Museum. The present writer has had the privilege of studying 12 individuals (7 females and 5 males) of what is obviously the same species. This supplementary material was taken from the same host species as was the type material, on February 27, 1901, by the Swedish Expedition to the White Nile and came to the present writer from the Riksmuseum in Stockholm through the courtesy of the late Prof. T. Odhner. On the basis of more adequate material the species has been re-examined. The original description has been found incomplete and in error in some respects. In the light of morphological evidences discovered in the present study, the species could not be accommodated within the genus *Rhadinorhynchus*, and even its allocation within the order Palaeacanthocephala cannot be justified.

The new name *Tenuisentis* is here applied to the generic concept based on *Rhadinorhynchus niloticus* Meyer as genotype. The species thus becomes designated as *Tenuisentis niloticus* (Meyer, 1932) Van Cleave, 1936.

In the original description of this species, Meyer gave no attention to the presence or absence of body spines. He was probably influenced in this direction by his study of *Rhadinorhynchus terebra*. According to Meyer (1932, p. 54) this last-named species is peculiar in that body spines are present on the female but lacking on the male. In arranging the species in his monograph, Meyer placed *R. terebra* and *R. niloticus* in juxtaposition, giving evidence that he regarded the spineless males of *R. niloticus* as parallel to the condition encountered in the males of *R. terebra*. The seven females before the present writer are wholly devoid of spines on the body. On this distinction alone the species *R. niloticus* could not be excluded from the family *Rhadinorhynchidae* unless previous workers have been in error in admitting *Leptorhynchoides* to this family, in spite of its lack of body spines. Other morphological characters of deeper phylogenetic significance are available as proof of the

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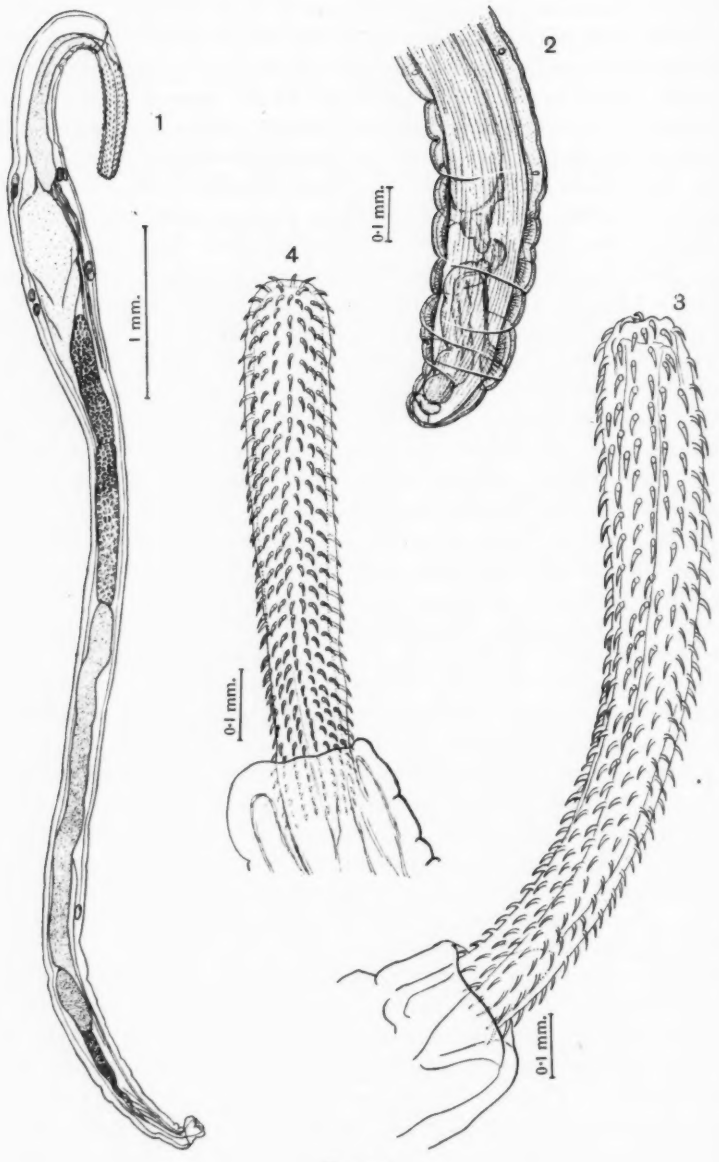
distinctness of *Tenuisentis* from the family Rhadinorhynchidae. These are as follows: (1) nature of the cement gland of the male; (2) form, number, and disposition of the subcuticular nuclei; (3) form and structure of the receptacle of the proboscis and its related structures; (4) the body cavities of the female and their relation to the genital tract; and (5) the form of the lemnisci. In the following section the morphological features will be discussed and their value in interpreting the relationships of *Tenuisentis niloticus* will be indicated.

(1) The cement gland (Fig. 1) in males of *Tenuisentis niloticus* is a single, elongated, syncytial mass, not divided into separate glands or follicles. For many years, this type of gland has been recognised as distinctive and wholly unlike the type ordinarily described. The alternative type is subject to great diversity of form and number of individual glands or follicles, but between these diverse forms and the syncytial cement glands there is no transitional condition. The syncytial glands therefore stand as a phylogenetically separate and distinct entity in the morphological series of cement glands, both quantitatively and qualitatively unlike the cement glands usually considered as typical for most members of the Acanthocephala. On the character of the cement gland alone *T. niloticus* would be definitely excluded from the genus *Rhadinorhynchus* and from the family for which it is type.

Among males of *Tenuisentis niloticus* available to the author, the cement gland ranges from 2·7 to 4·2 mm. in length. No specimen has been observed in which it reaches the length of 6 mm. recorded by Meyer in the original description of the species. The facts that Meyer refers to this organ in the plural and attributes the species to the genus *Rhadinorhynchus* give evidence that he was unaware of the true nature of the cement gland, thinking he was dealing with a multiple tubular system. It likewise seems probable that failure to differentiate between cement gland and other post-testicular organs of the male accounts for the excessive length which he recorded for the cement gland. Due to the histological condition of the material, the number, arrangement and degree of variability of the nuclei in the cement gland could not be determined. The nuclei are relatively large, and particularly prominent in the anterior end of the gland. In arrangement they seem to follow no definite pattern. In one particularly favourable whole mount there seems to be somewhere near 50 nuclei in the cement gland.

(2) The subcuticular nuclei (Fig. 1) likewise give evidence of the distinctness of *Tenuisentis* from the Rhadinorhynchidae. At least some of the subcuticular nuclei are giant nuclei arranged in distinctive, though not absolutely constant, pattern. The anterior region of the body contains 8 subcuticular nuclei disposed in 4 groups of 2 each. Four of these are near the posterior tip of the proboscis receptacle, 2 in the dorsal and 2 in the ventral body wall. Slightly posterior from this position 2 nuclei occur in the dorsal wall and 2 in the ventral. In whole mounts, nuclei of other regions of the subcuticula do not seem to conform to a set pattern. At least in some males a single nucleus appears in the ventral wall of the body near the posterior extremity



Figs. 1–4.

All figures are camera lucida drawings of stained whole mounts of *Tenuisentis niloticus*. The scale accompanying Fig. 1 has the value of 1 mm.; that with other drawings the value of 0·1 mm.

of the cement gland. In a number of instances, subcuticular nuclei in lateral position were observed in the posterior half of the body, but internal structures obscured this region to such an extent that conclusive evidences of presence or absence were lacking for most specimens.

Giant nuclei such as those here observed have considerable importance as indicators of possible relationships. The present author (Van Cleave, 1928) has shown how some genera may be arranged in phylogenetic series on characters provided by the subcuticular nuclei alone.

(3) *Proboscis receptacle.* Since the pioneer studies of Hamann (1892) the structure of the receptacle of the proboscis has been recognised as having deep significance in comparative morphology and hence in taxonomy. In the Rhadinorhynchidae the wall of the receptacle consists of two distinct muscular layers. In *Tenuisentis*, a single heavy muscular wall bounds the receptacle. On this feature alone the genus *Tenuisentis* would be excluded from the Rhadinorhynchidae and must seek its direct relationships among forms having a single-walled receptacle.

The receptacle (Fig. 1) is elongate, as is a natural accompaniment of a very long proboscis. The walls are unbroken except near the posterior extremity. One of the retractors of the receptacle penetrates the apical region of the posterior wall while the other emerges from the ventral wall slightly anterior to the extremity. Even under powerful illumination the retinacula and the brain within the receptacle have not been discernible.

(4) *Body cavities.* Several females of *Tenuisentis niloticus* give clear evidence of relations of the uterine bell to the body cavities as shown in Fig. 2. The uterus opens directly into the ventral chamber. According to Meyer's characterisations this condition is distinctive for the Archiacanthocephala, but the genus *Rhadinorhynchus* to which *Tenuisentis niloticus* was originally assigned falls into the order Palaeacanthocephala. The present writer has avoided this inconsistency by recognising a third order Eoacanthocephala to include *Tenuisentis* and 12 other genera. A full diagnosis of the order Eoacanthocephala is given in another paper now in the press.

(5) The lemnisci in the Rhadinorhynchidae are tubular, elongate, exceeding the length of the receptacle. In *T. niloticus* they are poorly defined in preserved specimens and seem to be shorter than the receptacle.

Observations which the present writer has made on the proboscis and its hooks are not in full agreement with those of Meyer. Few of the proboscides are fully everted in the material available for study. Most of these are about

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Legends to Figs. 1–4.

Fig. 1. General habitus drawing of entire male of *Tenuisentis niloticus* showing particularly the subcuticular nuclei and the male reproductive organs. The syncytial cement gland is the elongate region of uniform stippling immediately posterior to the testes.

Fig. 2. Posterior extremity of a female showing genital tract and its relations to the ligament sacs.

Fig. 3. Proboscis of a female showing number and arrangement of proboscis hooks.

Fig. 4. Proboscis of a male, partially retracted at base.

1 mm. long though some exceed this length (1·1 mm.), and a few are near the 0·75 mm. recorded by Meyer. While 16 is the usual number of longitudinal rows of hooks (Fig. 3), some individuals have a greater number. There are rarely less than 30 hooks in each longitudinal row, though Meyer has recorded 23. For some partially inverted proboscides as many as 38 or 40 hooks have been calculated for the individual rows. The hooks range from 30 to 41 μ in length over the greater part of the proboscis, though a few have a length as great as 47 μ.

No shelled embryos are present in any of the females examined, hence information on these remains lacking.

Observations made in this study serve as the basis for defining the genus *Tenuisentis* as follows:

Tenuisentis n.gen.

Generic diagnosis. Eoacanthocephala as defined by Van Cleave in a paper in press. Body long, slender, devoid of spines, with a slight enlargement of anterior extremity. Longitudinal canals of lacunar system chiefly dorsal and ventral but somewhat irregular in posterior region. Body wall with a few giant nuclei, chiefly dorsal and ventral in location but posteriorly at least some are lateral in position. Proboscis very long, slightly clavate, with numerous hooks. Receptacle with a single very thick muscular wall. Cement gland of male a much elongated syncytial mass. Ligament sacs of female distinctly separated, the uterine bell in direct communication with the ventral. Parasitic as adults in fishes; so far known only from the region of the Nile in Africa.

Genotype: *Tenuisentis niloticus* (Meyer 1932, p. 54) (= *Rhadinorhynchus niloticus*).

TENUISENTIDAE n.fam.

Since the genus *Tenuisentis* fails to agree with any family heretofore recognised, the writer proposes the recognition of a new family Tenuisentidae with the characters of the genus *Tenuisentis* as cited above.

Summary

Specimens collected by the Swedish Expedition to the White Nile in 1901 have been identified as *Rhadinorhynchus niloticus* Meyer, 1932. On the basis of this material, including the first recorded females, the species has been restudied. Points wherein the individuals are unlike the forms included in the family Rhadinorhynchidae are presented in detail. A new genus, *Tenuisentis*, is proposed with *Rhadinorhynchus niloticus* as type. This new genus becomes type of a new family, the Tenuisentidae, which is assigned to the order Eoacanthocephala.

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